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THE EVOLUTION OF THE CELL[1]

BY THE LATE PROFESSOR E. A. MINCHIN, F.R.S.

WHEN addressing an audience of biologists it would be superfluous to insist upon the importance of the study of the cell and its activities. It is now recognized almost universally that the minute corpuscles known by the somewhat unsuitable term "cells" are the vital units of which the bodies of animals and plants are built up, and that all distinctive vital processes—metabolism, growth and reproduction, sexual phenomena and heredity—reduce themselves ultimately to activities taking place in, and carried on by, the individual cells which build up the body as a whole. Each cell must be regarded as a living, individual organism which, however much it may be specialized for some particular function or form of vital activity, is capable of maintaining its life and existence in a suitable environment by carrying on all the necessary processes of metabolism which are the essential and distinctive characteristics of living beings. In the case of cells composing the complex body of the higher animals and plants the cells are mutually interdependent, and, with the exception of the mature germ-cells, can not maintain their existence apart from their fellows; that is to say, the only natural[2] environment suitable for their con-

[1] Address by the President to the Zoological Section of the British Association for the Advancement of Science. Manchester, 1915.

[2] It is not necessary to do more than refer here to the investigations that have been carried on in recent years with regard to the viability and multiplication of tissue-cells removed from the body in artificial culture-media.

tinued existence is the complex body or cell-commonwealth of which they form an integral part. But in the simplest forms of life the whole body of the living individual may reach no higher degree of complexity than the single cell, which is then seen as an organism physiologically complete in every respect, living a free and independent life in Nature and competing with other organisms of all kinds, simple or complex, in the universal struggle for existence amongst living beings. This statement of the "cell-theory" is that with which, I believe, the majority of modern biologists would agree; not without, however, some dissentients, amongst whom I personally am not to be numbered.[3]

The fundamental importance of the cell as a complete living organism, whether maintaining itself singly and independently or in union with other similar but individually specialized units, has made it the object of intensive and concentrated study, not only by those who group themselves according to their special points of view as zoologists, botanists, physiologists, etc., but also by a class of investigators who take the cell itself as the subject of a branch of biological investigation termed cytology, which deals with cells in a general manner independently of their provenance, whether animal or vegetable. Some knowledge of the cell and its activities is necessary at the present time for every one concerned with the study of living things, whether that study is pursued for its own sake and with disinterested objects, or with the intention of applying scientific principles to practical aims, as in medicine or agriculture. One might have expected, therefore, that at least some elementary understanding of the nature and significance of the cell, and the importance of cellular activities in the study of life and living things, would have formed at the present time an indispensable part of the stock of knowledge acquired by all intelligent persons who are ranked as "educated" in popular esti-

These experiments afford strong support to the view that the cell is to be regarded primarily as an independent living organism.

[3] See Appendix A.

mation. Unfortunately this is so far from being the case that it is practically impossible, in this country at least, to find any one amongst the educated classes to whom the words "cell" and "cytology" convey any meaning at all, except amongst those who have interested themselves specially in some branch of biology. Consequently, any discussion concerning the cell, although it may deal with the most elementary processes of life and the fundamental activities and peculiarities of living beings, ranks in popular estimation as dealing with some abstruse and recondite subject quite remote from ordinary life and of interest only to biological specialists. It must, however, be pointed out that the general state of ignorance concerning these matters is doubtless in great part due to the fact that an objective acquaintance with cells can not be obtained without the use of expensive and delicate optical instruments.

I propose in this address to deal with an aspect of cytology which appears to me not to have received as yet the attention which it deserves, namely, the evolution of the cell itself and of its complex organization as revealed by the investigation of cytologists. Up to the present time the labors of professed cytologists have been directed almost entirely towards the study of the cell in its most perfect form as it occurs in the Metazoa and the higher plants. Many cytologists appear indeed to regard the cell, as they know it in the Metazoa and Metaphyta, as the beginning of all things, the primordial unit in the evolution of living beings.[4] For my part I would as soon postulate the special creation of man as believe that the Metazoan cell, with its elaborate organization and its extraordinarily perfected method of nuclear division by karyokinesis, represents the starting-point of the evolution

[4] For example, my friend Dr. C. E. Walker, in an article in *Science Progress* (Vol. VII, p. 639), after stating that "The unit of living matter, so far as we know, is the cell," proceeds to deal with "that form in which it is found in the multicellular and the majority of unicellular organisms, both animal and vegetable" and then describes the typical cell of the cytologist, with nucleus, cytoplasm, centrosome, chrondriosomes, and reproduction with fully developed karyokinesis.

of life. So long, however, as the attention of cytologists is confined to the study of the cells building up the bodies of the higher animals and plants, they are not brought face to face with the stages of evolution of the cell, but are confronted only with the cell as a finished and perfected product of evolution, that is to say, with cells which, although they may show infinite variation in subordinate points of structure and activity, are nevertheless so fundamentally of one type that their plan of structure and mode of reproduction by division can be described in general terms once and for all in the first chapter of a biological text-book or in the opening lecture of a course of elementary biology.

One of the most striking features of the general trend of biological investigation during the last two decades has been the attention paid to the Protista, that vast assemblage of living beings invisible, with few exceptions, to the unassisted human vision and in some cases minute beyond the range of the most powerful microscopes of to-day. The study of the Protista has received in recent years a great stimulus from the discovery of the importance of some of the parasitic forms as invaders of the bodies of men and animals and causers of diseases often of a deadly nature; it has, however, yielded at the same time results of the utmost importance for general scientific knowledge and theory. The morphological characteristic of the Protista, speaking generally, is that the body of the individual does not attain to a higher degree of organization than that of the single cell. The exploitation, if I may use the term, of the Protista, though still in its initial stages, has already shown that it is amongst these organisms that we have to seek for the forms which indicate the evolution of the cell, both those lines of descent which lead on to the cell as seen in the Metazoa and Metaphyta, as well as other lines leading in directions altogether divergent from the typical cell of the text-book. We find in the Protista every possible condition of structural differentiation and elaboration, from cells

as highly organized as those of Metazoa or even, in some cases, much more so, back to types of structure to which the term cell can only be applied by stretching its meaning to the breaking-point. Already one generalization of cytologists has been torpedoed by the study of the Protista. The dictum "Omnis nucleus e nucleo" is perfectly valid as long as it is restricted to the cells of Metazoa and Metaphyta, to the material, that is to say, to which the professed cytologist usually confines his observations.[5] But in the Protista it is now well established that nuclei can arise *de novo,* not from preexisting nuclei but from the extranuclear chromatin for which Hertwig first coined the term "chromidia."

It is clear, therefore, that the results already gained from the study of the Protista have brought about a new situation which must be faced frankly and boldly. It is impossible any longer to regard the cell as seen in the Metazoa and as defined in the text-books as the starting-point of organic evolution. It must be recognized that this type of cell has a long history of evolution behind it, which must be traced out, so far as the data permit. The construction of phylogenies and evolutionary series is of course purely speculative, since these theories relate to events which have taken place in a remote past, and which can only be inferred dimly and vaguely from such fragments of wreckage as are to be found stranded on the sands of the time in which we live. Many important stages of evolution may be totally submerged and no longer available for study and consideration. The extent to which such speculations will carry conviction to a reasonable mind will depend entirely on the stores of

[5] Vejdovský ("Zum Problem der Vererbungsträger," Prag, 1911–1912, p. 120) has already maintained, for the cells of Metazoa, that Fleming's aphorism "Omnis nucleus e nucleo" should be changed to "Omnis nucleus e chromosomatis" [*sic*], on the ground that the nucleus, as such, is not an original cell-component "but is produced secondarily from the chromosomes of the mother-cell." If this is true, there is but little difference in detail, and none in principle, between the formation of "secondary" nuclei from chromidia and the reconstruction of a daughter-nucleus from chromosomes in the most perfected form of karyokinesis.

data that can be collected and which must be the last appeal for the cogency of all arguments and judgments. The study of the Protista is as yet in its infancy; groups have been recognized and have received ponderous designations, although their very existence is yet in doubt, as in the case of the so-called Chlamydozoa; and our knowledge of the affinities and mutual relationships of the groups is still very imperfect. All attempts, therefore, to trace the evolution of the Protista must be considered as purely tentative at present. If I venture upon any such attempt, it is to be regarded as indicating a firm belief on my part that the evolution of the cell has taken place amongst the Protista, and that its stages can be traced there, rather than as a dogmatic statement that the evolution has taken place in just the manner which seems to me most probable. When we reflect on the irreconcilable differences of opinion amongst zoologists with regard to the origin and ancestry of vertebrates, for example, we may well be cautious in accepting pedigrees in Protista.

Before, however, I can proceed to deal with my main subject, it is absolutely necessary that I should define clearly the sense in which I propose to use certain terms, more especially the words "cell," "nucleus," "chromatin," "protoplasm" and "cytoplasm." Unless I do so my position is certain to be misunderstood, as, indeed, it has been already by some of my critics.

The term cell was applied originally by botanists to the single chambers or units of the honeycombed structure seen in the tissues of plants. The application of the term to such structures is perfectly natural and intelligible, since each such cell in its typical form is actually a closed space limited by firm walls, and containing a relatively large quantity of fluid cell-sap and a small quantity of the slimy protoplasmic substance. When these structures were first discovered, the limiting membrane or wall of the cell was regarded as essential, and less importance was attached to its contents. With increased knowledge,

however, and especially when animal tissues came to be studied, it became apparent that the cell-wall, like the fluid cell-sap, was a secondary product, and that the essential and primary part of the cell was the viscid protoplasmic substance, in which a peculiar body, the "nucleus," or kernel, was found to be universally present. Consequently the application and meaning of the term cell had to undergo an entire change, and it was defined as a small mass or corpuscle of the living substance, protoplasm, containing at least one nucleus. To these essential constituents other structures, such as a limiting membrane or cell-wall, and internal spaces—vacuoles—filled with watery fluid, might be added as products of the secretory or formative activity of the living substance; but such structures were no longer regarded as essential to the definition of the cell, since in many cases they are not present. It is to be regretted in some respects that with this changed point of view the term "cell," used originally under a misapprehension, was not replaced by some other term of which the ordinary significance would have been more applicable to the body denoted by it.[6]

The chief point that I wish to establish, however, is that the term cell was applied originally to the protoplasmic corpuscles building up the bodies of the Metazoa and Metaphyta, each such corpuscle consisting of a minute individualized mass of the living substance and containing a nucleus. Hence a complete cell is made up of two principal parts or regions, the nucleus and the remainder of the protoplasmic body, termed the cytoplasm. By some authors the term protoplasm is restricted to the cytoplasmic portion of the cell, and protoplasm is then contrasted with nucleus; but it is more convenient to consider the whole cell as composed of protoplasm divided into two regions, nucleus and cytoplasm.

We come now to the consideration of the body termed

[6] "Nothing could be less appropriate than to call such a body a 'cell'; yet the word has become so firmly established that every effort to replace it by a better has failed, and it probably must be accepted as part of the established nomenclature of science."—E. B. Wilson, "The Cell," p. 19.

the nucleus, which undoubtedly possesses an importance in the life and functions of the cell far greater than would be inferred from the name given to it. A nucleus, as seen in its typical form, has a limiting membrane enclosing a framework composed of a substance termed "linin." The framework has the form of a network, which is probably to be interpreted, primitively at least, as the optical expression of an alveolar structure similar to that seen also in the cytoplasm, but of coarser texture, and the apparent "threads" of the linin-framework may then be the optical sections of the partitions between neighboring alveoli. Such an interpretation does not exclude the possibility of the formation of real threads or fibers in the framework in certain cases or during particular periods of nuclear activity; just as fibrous structures may arise in the alveolar cytoplasm also. The cavities of the framework contain a watery fluid or nuclear sap, probably of the same nature as the fluid enchylema or cell-sap contained in the alveolar framework of the cytoplasm. At the nodes of the alveolar framework are lodged grains or masses of *chromatin,* a substance which must engage our most particular attention, since it is the essential constituent of the nucleus, universally present in all nuclei, whether of the simplest or of the most complex types. In addition to the chromatin-grains, which are distributed in various ways over the linin-framework, there are to be found usually one or more masses termed nucleoli, composed of a material which differs from chromatin in its reactions and has been termed plastin.

In the foregoing paragraph I have described in general terms the typical nucleus of the text-books, as found commonly in the cells that build up the bodies of ordinary animals and plants. The minutiæ of the details of structure and arrangement of the constituent parts may vary infinitely, but the type remains fairly constant. When we come, however, to the nuclei of the Protista, such pronounced modifications and variations of the type are met with that a description in general terms is no longer pos-

sible. I shall deal with some of these types later in my attempts to reconstruct the evolution and phylogeny of the cell. I will draw attention now only to a few salient points. In the Protist cell the chromatin is not necessarily confined to the nucleus, but may occur also as extranuclear grains and fragments termed chromidia, scattered through the protoplasmic body; and the chromatin may be found only in the chromidial condition, a definite nucleus being temporarily or permanently absent. Further, when a true nucleus is present in the Protist body, it seldom contains a nucleolus of the same type as that seen in the nuclei of tissue-cells, that is to say, a mass of pure plastin, but in its place is found usually a conspicuous body which shows reactions agreeing more or less closely with those of chromatin and which consists of a plastin-basis more or less densely impregnated with chromatin. Such a body is termed a karyosome (or chromatin-nucleolus) to distinguish it from the true nucleoli (plastin-nucleoli) characteristic of tissue-cells. According as the plastin or the chromatin predominates in the composition of a karyosome, its reactions may resemble more nearly those of a true nucleolus in the one case, or those of chromatin in the other. The so-called karyosomatic type of nucleus is very common in the Protista, but by no means of invariable occurrence; in many cases the nucleus consists of a clump of small grains of chromatin, with no distinct karyosome, or with a karyosome which consists mainly of plastin. Thus two extreme types of nuclear structure can be distinguished and may be termed provisionally the karyosomatic type and the granular type, ignoring for the sake of convenience in nomenclature the types of structure transitional between the two; as, for example, types in which a distinct karyosome is seen together with more or fewer peripherally arranged grains of chromatin.

In either the karyosomatic or the granular type of Protist nucleus we may find great simplification of the complex type of nuclear structure seen in the tissue-cells

of animals and plants. Thus in the first place a distinct nuclear membrane may be entirely absent and the chromatin-elements, whether occurring in the form of a compact karyosome or of a clump of grains, are lodged simply in a vacuole in the cytoplasm, that is to say in a cavity containing a watery fluid of nuclear sap in which the mass or masses of chromatin are suspended. It is a moot point, to which I shall return again, whether in nuclei of this simple type the linin-framework may sometimes be absent altogether, or whether it is invariably present in at least a rudimentary form, appearing as delicate threads (in optical section) extending from the chromatin-masses to the limiting wall of the nuclear vacuole, or between the grains of chromatin themselves. When such a framework can be detected, the nucleus acquires the appearance, in preserved preparations at least, of possessing a definite structure and is often termed a resting nucleus; many observations have shown, however, that the nucleus during life is undergoing continual internal movements and re-arrangements of its parts and is by no means at rest. The linin-framework can not, therefore, be regarded in any way as a rigid skeleton, but must be interpreted as an alveolar framework similar to that of the general protoplasm and equally liable to movement, displacement and change.

From this survey, necessarily most brief and superficial, of the manner in which the nuclei of Protists may vary from the type of nucleus described in the text-books, it is at once evident that the essential part of the nucleus is the chromatin, and that the other structural constituents of the nucleus, namely, membrane, framework, and plastin or nucleolar bodies, are to be regarded as accessory components built up round, or added to, the primary nuclear material, the chromatin. Even with regard to the nuclei of Metazoa it is maintained by Vejdovsky that at each cell-generation the entire nucleus of the daughter-cell is produced from the chromosomes alone of the

mother cell.[7] The simplest body which can be recognized as a nucleus, distinct from the chromidia scattered without order or arrangement throughout the protoplasmic body, is a mass of chromatin or a clump of chromatin-grains supported on a framework and lodged in a special vacuole in the cytoplasm. The complexity seen in the most perfect type of nucleus takes origin by progressive elaborations of, and additions to, a structure of this simple and primitive type.

This brings me to a point which I wish to emphasize most strongly, namely, that the conception of a true cell-nucleus is essentially a structural conception. A nucleus is not merely an aggregation of chromatin; it is not simply a central core of some chemical substance or material differing in nature from the remainder of the protoplasm. As Dobell has well expressed it, a pound of chromatin would not make a nucleus. The concepts "nucleus" and "chromatin" differ as do those of "table" and "wood." Although chromatin is the one universal and necessary constituent entering into the composition of the cell-nucleus, a simple mass of chromatin is not a nucleus.[8] A true nucleus is a cell-organ, of greater or less structural complexity, which has been elaborated progressively in the course of the evolution of the cell;

[7] Walker, on the other hand, considers that "it seems quite possible that the chromatin is merely a secretion of the linin." (*Science Progress*, Vol. VII, p. 641.) I doubt whether there are many cytologists who would admit this possibility, and I think that very few protistologists would assent to any such notion, since in the nuclei of Protista the linin-framework is in many cases very little in evidence, if present at all.

[8] Professor Armstrong writes: "Every organism must possess some kind of nucleus, visible or invisible: some formative center round which the various templates assemble that are active in directing the growth of the organism." (*Science Progress*, Vol. VII, p. 328.) I need hardly point out that a chemical nucleus of this kind is not in the least what the biologist or cytologist means by the term cell-nucleus. The one is a subjective postulate necessary for the comprehension of the activities of any speck of living matter or any portion, however minute, of a living organism; the other is a concrete structure, known to us by actual observation, and as much an integral part of the true cell, considered as a definite type of organism, as a backbone or its morphological equivalent is essential to the definition of a true vertebrate.

it is as much an organ of the cell as the brain is an organ of the human body. As a definite cell-organ, it performs in the life and economy of the cell definite functions, which it is the province of the cytologist to observe and to study, and if possible to elucidate and explain. As an organ of the cell, however, it has no homologue or analogue in the body of the multicellular animals or plants; there is no organ of the human body, taken as a whole, similar or comparable to the nucleus of the cell. Consequently, in studying the functions of the nucleus the human cytologist finds himself in the same difficult position that an intelligent living being lacking the sense of sight would be when trying to discover the function of visual organs in other organisms possessing that sense. There is no organ of known and understood functions with which the cytologist can compare the cell-nucleus directly.

The foregoing brief consideration of the nucleus leads me now to discuss in more detail the nature and properties of the essential nuclear substance, the so-called chromatin. To define, or characterize adequately, this substance is a difficult task. The name chromatin is derived from the fact that this substance has a peculiar affinity for certain dyes or stains, so that when a cell is treated with the appropriate coloring reagents—with so-called nuclear stains—the chromatin in the nucleus stands out sharply, by reason of being colored in a different manner from the rest of the cell. In consequence, the statement is frequently made, in a loose manner and without reflection, that chromatin is recognized by its staining reactions, but in reality this is far from being true. When a preparation of an ordinary cell is made by the methods of technique commonly in use, the chromatin is recognized and identified by its position in a definite body with characteristic structure and relations to the cell as a whole, namely the nucleus, and this is equally true whether the chromatin has been stained or not. When

the cell has been stained with one of the dyes ordinarily in use for coloring the chromatin, there are often seen in the cytoplasm grains that are colored in exactly the same manner as the chromatin-grains lodged in the nucleus. Is an extranuclear grain which stains like chromatin to be identified, *ipso facto,* as chromatin? By no means; it may or it may not be chromatin. Simple inspection of a stained preparation is altogether inadequate to determine whether such a body is or is not chromatin. Any so-called chromatin-stain colors many bodies which may occur in a cell besides the chromatin, and it may be necessary to try a great many different stains before a combination is found which will differentiate a given cytoplasmic enclosure from a true chromatin-grain by its color-reactions. The so-called volutin-grains, for example, which are found commonly in the cytoplasm of many Protists, are identified by the fact that they have a stronger affinity for "chromatin-stains" than chromatin itself.

When, moreover, chromatin is compared with regard to its staining-reactions, both in different organisms, and in the same organism at different times, it is found to react very differently to one and the same stain. A striking example of this capriciousness is seen when a preserved film is made of the blood of some vertebrate which has nucleated blood-corpuscles, such as a bird or fish, and which contains also parasitic trypanosomes. It is easy to stain the nuclei of the blood-corpuscles with various stains, as, for example, carmine-stains such as picro-carmine or alum-carmine, which will not color the nuclei of the trypanosomes in the slightest. Moreover, every cytologist knows that the "chromaticity" of the chromatin varies enormously in different phases of the nuclear cycle of generation; it is often difficult to stain the chromatin in the "resting" nucleus, but the first sign of impending nuclear division is a marked increase in the staining powers of the chromatin. There is no dye known which can be relied upon to stain chromatin always, or wherever

it occurs. Methyl-green has been claimed to be the most reliable and certain of nuclear stains, but R. Hertwig, in his classical researches upon *Actinosphærium*, showed that it sometimes fails to stain chromatin. It is perfectly conceivable that there might be varieties of chromatin which could not be stained by any dye whatsoever.

I have felt bound to insist strongly upon the inadequacy of staining-methods for the detection and identification of chromatin, well known though these facts are to every cytologist, because here also I note a tendency amongst biological chemists to regard staining-properties as the sole criterion of chromatin. In reality such properties are of entirely secondary importance. To use the terminology of formal logic, staining-properties are an "accident," though it may be an "inseparable accident," of chromatin, not a "difference" which can be used to frame a logical definition, *per genus et differentias,* of this substance. If chromatin were nothing more than "stainable substance," as Professor Armstrong terms it,[9] some of the most important results of cytological investigation would be deprived of all real significance and reduced to the merest futilities.

What then is the true criterion of the chromatin-substance of living organisms? From the chemical point of view the essential substance of the cell-nucleus would appear to be characterized by a complexity of molecular structure far exceeding that of any other proteins, as well as by certain definite peculiarities. Especially characteristic of chromatin is its richness in phosphorus-compounds, and it stands apart also from other cell-elements in its solvent reactions, for example, resistance to peptic digestion. E. B. Wilson, in his well-known treatise, has emphasized the "cardinal fact . . . that there is a definite and constant contrast between nucleus and cytoplasm." The outstanding feature of the nucleus is the constant presence in abundance of nuclein and nucleoproteins. Nuclein, which is probably identical with chromatin, is a

[9] *Science Progress,* Vol. VII, p. 327.

complex albuminoid substance rich in phosphorus. It is the phosphorus-content of chromatin that is its most characteristic chemical peculiarity as contrasted with the cytoplasm. How far these features are common, however, to all samples of chromatin in all types of living organisms universally, can not, I think, be stated definitely at present; at any rate, it is not feasible for a cytologist of these days to identify a granule in a living organism or cell as chromatin solely by its chemical reactions, although it is quite possible that at some future time purely chemical tests will be decisive upon this point—a consummation devoutly to be wished.

The only criterion of chromatin that is convincing to the present-day biologist is the test of its behavior, that is to say, its relations to the life, activity and development of the organism. I may best express my meaning by objective examples. If I make a preparation of *Arcella vulgaris* by suitable methods, I see the two conspicuous nuclei and also a ring of granules lying in the cytoplasm, stained in the same manner as the chromatin of the nuclei. Are these extranuclear granules to be regarded also as chromatin? Yes, most decidedly, because many laborious and detailed investigations have shown that from this ring of granules in *Arcella* nuclei can arise, usually termed "secondary" nuclei for no other reason than that they arise *de novo* from the extranuclear chromatin and quite independently of the "primary" nuclei. The secondary nuclei are, however, true nuclei in every respect, as shown by their structure, behavior and relations to the life-history of the organism; they may fuse as nuclei of gametes (pronuclei) in the sexual act and they become, with or without such fusion, the primary nuclei of future generations of *Arcella;* they then divide by karyokinesis when the organism reproduces itself in the ordinary way by fission, and are replaced in their turn by new secondary nuclei at certain crises in the life-history. In view of these facts it can be asserted without hesitation that the ring of staining granules in *Arcella* is composed of, or at

least contains, true chromatin-grains, extranuclear chromatin for which R. Hertwig's term chromidia is now used universally. It is interesting to note that until the life-history of *Arcella* was studied in recent times the conspicuous ring of chromidia was generally overlooked and is not shown in some of the older pictures of the organism.

If, on the other hand, I make a preparation of some unidentified amœba occurring casually in pond-water or in an infusion, and find in its cytoplasm certain grains staining in same manner as the chromatin of the nucleus, it is quite impossible, without a knowledge of the life-history of the organism, to assert definitely that the grains in question are or are not true chromidia. They might equally well turn out to be volutin or any other substance that has an affinity for the particular chromatin-stains used in making the preparation.

The fact that at the present time the only decisive criterion of what is or is not chromatin is supplied only by its behavior in the life-history and its relation to the organism, makes it much easier to identify the chromatin in some cases than in others. In those Protista or cells which contain, during the whole or a part of the life-history, one or more true nuclei, recognizable as such unmistakably by their structure and their characteristic relations to the reproductive and sexual phenomena of the organism, the chromatin can be identified with certainty. If chromidia occur in the cell-body in addition to true nuclei or even if the nuclei are temporarily absent during certain crises of the life-history and the chromatin occurs then only in the form of chromidia, there is still no difficulty in identifying the scattered chromatin-grains by the fact that they contribute, soon or later, to the formation of nuclei.

On the other hand, in the simplest Protist organisms which do not contain definite, compact nuclei recognizable by their structure and behavior, the identification of the chromatin may become correspondingly difficult. In the absence of definite chemical criteria the term chromatin

acquires then a greater or less degree of vagueness and uncertainty of application, and it is not easy to avoid a tendency to a *petitio principii* in attempting to define or identify it. To a large extent we are thrown back upon the staining-reactions, which I have already shown to be very unreliable, backed up by analogies with those forms which possess definite nuclei. Since in the cells of all animals and plants, and in all Protista which possess a true nucleus, the chromatin is the one constituent which is invariably present, as I shall point out in more detail subsequently, there is at least a strong presumption, though not of course amounting to absolute proof, that it is present, or at least is represented by some similar and genetically homologous constituents, in the forms of simpler structure also. If then in Protista of primitive type we find certain grains which exhibit the characteristic staining-reactions of chromatin to be constantly present in the organism, grains which grow and divide as a preliminary to the organism multiplying by fission and which are partitioned amongst the daughter-organisms during the process of fission, so that each daughter-individual reproduces the structure of the parent-form from which it arose; then there is very strong *prima facie* evidence, to say the least, for regarding such grains as homologous with the chromatin-grains of ordinary cells.

Having now defined or explained, as well as I am able, the terms of which I am about to make use, I return to my main theme, the cell and its evolution. To summarize the points already discussed, a typical cell is a mass of protoplasm differentiated into two principal parts or regions, the cytoplasm and the nucleus, or, it may be, two or more nuclei. The cytoplasm may or may not contain chromatin-grains in addition to other enclosures, and may possess cell-organs of various kinds. The nucleus, highly variable in minute structure, possesses one invariable constituent, the chromatin-material in the form of grains and masses of various sizes.

The cell, therefore, in its complete and typical form, is

an organism of very considerable complexity of structure and multiplicity of parts. The truth of this proposition is sufficiently obvious even from simple inspection of the structural details revealed by the microscope in cells in the so-called "resting condition," but still more so from a study of their activities and functions. The vital processes exhibited by the cell indicate a complexity of organization and a minuteness in the details of its mechanism which transcend our comprehension and baffle the human imagination, to the same extent as do the immensities of the stellar universe. If such language seems hyperbolic, it is but necessary to reflect on some of the established discoveries of cytology, such as the extraordinary degree of complication attained in the process of division of the nucleus by karyokinesis, or the bewildering series of events that take place in the nuclei of germ-cells in the processes of maturation and fertilization. Such examples of cell-activity give us, as it were, a glimpse into the workshop of life and teach us that the subtlety and intricacy of the cell-microcosm can scarcely be exaggerated.

On the assumption that an organism so complex and potent was not created suddenly, perfect and complete as it stands, but arose, like all other organisms, by progressive evolution and elaboration of some simpler form and type of structure, it is legitimate to inquire which of the various parts of the cell are the older and more primitive and which are more recent acquisitions in the course of evolution. But it must be clearly pointed out, to start with, that the problem posed in such an inquiry is perfectly distinct from, and independent of, another point which has often been discussed at length, namely, the question whether any parts of the cell, and if so which parts, are to be regarded as "living" or "active" in distinction to other parts which are to be regarded as "not-living" or "passive." This discussion, in my opinion, is a perfectly futile one, of which I intend to steer clear.

We may agree that in any given cell or living organism,

simple or complex in structure, all the parts are equally "living" and equally indispensable for the maintenance of life, or at least for the continuance of the vital functions in the normal, specific manner, without losing the right to inquire which of those parts are the phylogenetically older. A simple analogy will serve to point my meaning. A man could not continue to live for long if deprived either of his brain, his digestive tract, his lungs, his heart, or his kidneys, and each of these organs is both "living" in itself and at the same time an integral part of the entire organization of the human body; yet no one would think of forbidding comparative anatomists to discuss, from the data at their command, which of these organs appeared earlier, and which later, in the evolution of the phylum Vertebrata. Moreover, speculative though such discussions must necessarily be, there is no one possessing even a first-year student's knowledge of the facts who would controvert the statement that the digestive tract of man is phylogenetically older than the lung. Speculative conclusions are not always those that carry the least conviction.

The evolution of the cell may be discussed as a morphological problem of the same order as that of the phylogeny of any other class or phylum of living beings, and by the same methods of inquiry. In the first place there is the comparative method, whereby different types of cell-structure can be compared with one another and with organisms in which the cell-structure is imperfectly developed, in order to determine what parts are invariable and essential and what are sporadic in occurrence and of secondary importance, and if possible to arrange the various structural types in one or more evolutionary series. Secondly there is the developmental or ontogenetic method, the study of the mode and sequence of the formation of the parts of the cell as they come into existence during the life-history of the organism. Both these methods, which are founded mainly on observation, require to be checked and controlled by the experimental

methods of investigating both the functions and behavior of the organism and of its parts.

So long as cytologists limit their studies to the cells building up the tissues of the higher animals and plants, the comparative method has a correspondingly limited scope, and that of the ontogenetic method is even more restricted. Both methods receive at once, however, an enormously extended range when the Protista are taken into consideration. Then, moreover, we see the dawning possibility of another method of investigation, that, namely, of the chemical evolution of the organisms. Already some of the simpler Protista, the Bacteria, are characterized and classified largely by their chemical activities; but in more complex organisms, in those which have attained complete cell-structure, such as Protozoa, the data of chemistry do not as yet supply the evolutionist with a helpful method of investigation.

The problem of cell-evolution may be attacked by the help of the methods outlined in the foregoing remarks, beginning with the consideration of the primary structural differentiation of the typical cell, the distinction of nucleus, or rather chromatin, and cytoplasm. Since all cells known to us exhibit this differentiation, we have three possibilities as regards the manner in which it has come about, which may be summarized briefly as follows: either the cytoplasmic and chromatinic constituents of the cell have arisen as differentiations of some primitive substance, which was neither the one nor the other; or one of these two substances is a derivative of the other, in the course of evolution, either cytoplasm of chromatin, or chromatin of cytoplasm.

The idea of a primitive, undifferentiated protoplasmic substance was first put forward by Haeckel, who employed for it the term "plasson" invented by Van Beneden[10] to denote "la substance constitutive du corps des Monères et des cytodes . . . le substance formative par

[10] *Bull. de l'Acad. Roy. de Belgique*, Second Series, Vol. XXXI (1871), p. 346.

excellence." The simplest elementary organisms were not cells, but cytodes, "living independent beings which consist entirely of a particle of plasson; their quite homogeneous or uniform body consists of an albuminous substance which is not yet differentiated into karyoplasm and cytoplasm, but possesses the properties of both combined."[11] It is emphasized[12] that a sharp distinction must be drawn between protoplasm and plasson, the latter being a homogeneous albuminous formative substance ("Bildungsstoff") corresponding to the "Urschleim" of the older nature-philosophy.

Haeckel, as was usual with him, did not content himself with putting forward his ideas as abstract speculations, but sought to provide them with a concrete and objective foundation by professing to have discovered, and describing in detail, living and existing organisms which were stated to remain permanently in the condition of cytodes. In consequence, a purely speculative notion was permitted to masquerade for many years under the false appearance of an objective phenomenon of nature, until the error was discovered gradually and the phantom banished from the accepted and established data of biology. Organisms supposed to be of the nature of cytodes constituted Haeckel's systematic division, Monera, of which there were supposed to be two subdivisions, the Phytomonera and the Zoomonera. The Phytomonera were stated to have the plasson colored green and to live in a plant-like manner; the Zoomonera were colorless amœboid masses of plasson which nourished themselves in the animal manner. The Bacteria were also included by Haeckel in his Monera, apparently, or at all events ranked as cytodes.[13] Most importance, however, was attributed by Haeckel to the large amœboid forms of Monera, described as without nuclei or contractile vacuoles, but as representing simply structureless

[11] *Anthropogenie*, sixth edition, Leipzig, 1910, p. 119.
[12] *Ibid.*, p. 532.
[13] *Ibid.*, p. 119.

contractile masses of albumin ("Eiweiss"), perfectly homogeneous;[14] examples of these were announced to exist under the names "Protamœba" and "Protogenes," denoting forms of life which Haeckel claimed to have discovered, but which have never been found again by any other naturalist. These organisms, as described by Haeckel, were by no means such as the modern microscopist would call minute; on the contrary, they were relatively large, and some of the forms added to the Monera by Haeckel's contemporaries might even be termed gigantic, as, for example, the supposed organism *Bathybius,* discovered in the bottles of the *Challenger* Expedition, which was believed to cover large areas of the floor of the ocean with a layer of primordial protoplasm, but which proved finally to be a precipitation by alcohol of the gypsum in sea-water.

The theory of plasson and of the cytodes of Haeckel may be considered first from the purely speculative standpoint of the origin of the living substance, a problem with which I wish to become entangled here as little as possible, since it is my object to confine myself so far as possible to deductions and conclusions that may be drawn from known facts and concrete data of observation and experiment. If, however, we postulate a chemical evolution of protoplasm, and believe that every degree of complexity exists, or at least has existed, between the simplest inorganic compounds and the immensely complicated protein-molecules of which the living substance is composed, then no doubt chemical compounds may have existed which in some sense were intermediate in their properties between the two constituents, cytoplasm and chromatin, found in all known samples of the living substance of organisms. In this sense and on such a hypothesis, a substance of the nature of plasson may perhaps be recognized or postulated at some future time by the biochemist, but this is a subject which I am quite incompetent to discuss. To the modern biologist, who can deal

[14] See his "Prinzipien der generellen Morphologie," Berlin, 1906, p. 61.

only with living things as he knows them, Haeckel's plasson must rank as a pure figment of the imagination, altogether outside the range of practical and objective biology at the present time. All visible living things known and studied up to the present consist of protoplasm, that is to say, of an extremely heterogeneous substance of complex structure, and no living organism has been discovered as yet which consists of homogeneous structureless albuminous substance. Van Beneden, who is responsible for the word plasson, though not for the cytode-theory, was under the impression that he had observed a non-nucleated homogeneous cytode-stage in the development of the gregarine of the lobster, *Gregarina* (*Porospora*) *gigantea*. Without entering into a detailed criticism of Van Beneden's observations upon this form, it is sufficient to state that the development of gregarines is now well known in all its details, and that in all phases of their life-cycle these organisms show the complete cell-structure, and are composed of nucleus and cytoplasm. Moreover, all those organisms referred by Haeckel to the group Monera which have been recognized and examined by later investigators have been found to consist of ordinary cytoplasm containing nuclei or nuclear substance (chromatin). In the present state of biological knowledge, therefore, the Monera as defined by Haeckel must be rejected and struck out of the systematic roll as a non-existent and fictitious class of organisms.

Since no concrete foundation can be found for the view that cytoplasm and chromatin have a common origin in the evolution of living things, we are brought back to the view that one of them must have preceded the other in phylogeny. The theories of evolution put forward by Haeckel and his contemporaries, if we abolish from them the notion of plasson and substitute for it that of ordinary protoplasm, would seem to favor rather the view that the earliest forms of life were composed of a substance of the nature rather of cytoplasm, and that the nuclear substance or chromatin appeared later in evolu-

tion as a product or derivative of the cytoplasm. I have myself advocated a view diametrically opposite to this, and have urged that the chromatin-substance is to be regarded as the primitive constituent of the earliest forms of living organisms, the cytoplasmic substance being a later structural complication. On this theory the earliest form of living organism was something very minute, probably such as would be termed at the present day "ultra-microscopic." After I had urged this view in the discussion on the origin of life at the Dundee Meeting of the British Association in 1912 a poem appeared in *Punch*,[15] dividing biologists into "cytoplasmists" and "chromatinists." I must confess myself still a whole-hearted chromatinist. But before I consider this point I may refer briefly to some other speculations that have been put forward with regard to the nature of the earliest form of life. It is manifestly quite impossible that I should undertake here to review exhaustively all the theories and speculations with regard to the origin of life and the first stages in its evolution that have been put forward at different times. I propose to limit myself to the criticism of certain theories of modern times which, recognizing the fundamental antithesis between chromatin and cytoplasm, regard these two cell-constituents as representing types of organisms primitively distinct, and suggest the hypothesis that true cells have arisen in the beginning as a process of symbiosis between them. Boveri, whose merits as a cytologist need no proclamation by me, was the first I believe to put forward such a notion; he enunciated the view that the chromosomes were primitively independent elementary organisms which live symbiotically with protoplasm, and that the organism known as the cell arose from a symbiosis between two kinds of simple organisms, "Monera."[16]

A similar idea lies at the base of the remarkable and

[15] Vol. CXLIII, p. 245.

[16] *Fide* Vejdovsky, *l. c.* I have not had access to the work of Boveri, in which he is stated to have put forward these ideas.

ingenious speculations of Mereschkowsky,[17] who assumes a double origin for living beings from two sorts of protoplasm, supposed not only to differ fundamentally in kind but also to have had origins historically distinct. The first type of protoplasm he terms mycoplasm,[18] which is supposed to have come into existence during what he calls the third epoch[19] of the earth's history, at a time when the crust of the earth had cooled sufficiently for water to be condensed upon it, but when the temperature of the water was near boiling-point; consequently the waters of the globe were free from oxygen, while saturated with all kinds of mineral substances. The second type of protoplasm was amœboplasm, the first origin of which is believed to have taken place during a fourth terrestrial epoch when the waters covering the globe were cooled down below 50° C., and contained dissolved oxygen but fewer mineral substances. Corresponding with the differences of the epoch and the conditions under which they arose, Mereschkowsky's two types of protoplasm are distinguished by sharp differences in their nature and constitution.

Mycoplasm, of which typical examples are seen in bacteria, in the chromatin-grains of the nucleus and the chromatophores of plant-cells, is distinguished from amœboplasm, which is simply cytoplasm, by the following points. (1) Mycoplasm can live without oxygen, and did so in the beginning at its first appearance when the temperature of the hydrosphere was too high for it to have contained dissolved oxygen; only at a later period, when the temperature became low enough for the water to contain oxygen in solution, did some of the forms begin

[17] Mereschowsky, C., "Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen," *Biol. Centralblatt*, XXX, 1910, pp. 278–303, 321–347, 353–367.

[18] The term mycoplasm used by Mereschowsky must not be confounded with the similar word used by Eriksson and other botanists in reference to the manner in which Rust-Fungi permeate their hosts.

[19] In the first epoch the earth was an incandescent mass of vapor; in the second it had a firm crust, but the temperature was far too high to permit of the condensation of water-vapor upon its surface.

to adapt themselves to these conditions, and became secondarily facultative or obligate aerobes. Amœboplasm, on the other hand, can not exist without a supply of oxygen. (2) Mycoplasm can support temperatures of 90° C. or even higher; amœboplasm can not support a temperature higher than 45° C. or 50° C. (3) Mycoplasm is capable of building up albumins and complex organic substances from inorganic materials; amœboplasm is incapable of doing so, but requires organic food. (4) Mycoplasm has restricted powers of locomotion and is incapable of amœboid movement, or of forming the contractile vacuoles seen commonly in amœboplasm. (5) Mycoplasm, in contrast to amœboplasm, is rich in phosphorus and nuclein. (6) Mycoplasm is extraordinarily resistant to poisons and utilizes as food many substances that are extremely deadly to amœboplasm, such as prussic acid, strychnine and morphia. (7) Amongst minor differences, mycoplasm is characterized by the presence of iron in the combined state and possesses a far more complicated structure than amœboplasm, a peculiarity which enables mycoplasmic cell-elements (chromosomes) to function as the bearers of hereditary qualities.

The course of the evolution of living beings, according to Mereschkowsky, was as follows. The earliest forms of life were "Biococci," minute ultra-microscopic particles of mycoplasm, without organization, capable of existing at temperatures near boiling-point and in the absence of oxygen, possessing the power of building up proteins and carbohydrates from inorganic materials, and very resistant to strong mineral salts and acids and to various poisons. From the Biococci arose in the first place the Bacteria, which for a time were the only living inhabitants of the earth. Later, when the temperature of the terrestrial waters had been lowered below 50° C., and contained abundant organic food in the shape of Bacteria, amœboplasm made its appearance in small masses as non-nucleated Monera which crept in an amœboid manner on the floor of the ocean and devoured Bacteria.

The next step in evolution is supposed to have been that, in some cases, micrococci ingested by the Monera resisted digestion by them and were enabled to maintain a symbiotic existence in the amœboplasm. At first the symbiotic micrococci were scattered in the Moneran body, but later they became concentrated at one spot, surrounded by a membrane, and gave rise to the cell-nucleus. In this way, by a "symbiogenesis" or process of symbiosis between two distinct types of organisms, Mereschkowsky believes the nucleated cell to have arisen, an immense step forward in evolution, since the locomotor powers of the simple and delicate Monera were now supplemented by the great capability possessed by the Bacteria of producing ferments of the most varied kinds.

Meanwhile it is supposed that the free Bacteria continued their natural evolution and gave rise to the Cyanophyceæ, and to the whole group of Fungi. The plant-cell came into existence by a further process of symbiogenesis, in that some of the Cyanophyceæ, red, brown or green in color, became symbiotic in nucleated cells, for the most part flagellates, in which they established themselves as the chromatophores or chlorophyll-corpuscles. In this way Mereschkowsky believes the vegetable cell to have come into existence, and the evolution of the Vegetable Kingdom to have been started, as a double process of symbiosis. Those amœboid or flagellated organisms, on the other hand, which formed no symbiosis with Cyanophyceæ, continued to live as animals and started the evolution of the Animal Kingdom.

As a logical deduction from this theory of the evolution of living beings, Mereschkowsky classifies organisms generally into three groups or Kingdoms: first the Mycoidea, comprising Bacteria, Cyanophyceæ, and Fungi, and in which no symbiosis has taken place; secondly, the Animal Kingdom, in which true cells have arisen by a simple symbiosis of mycoplasm (chromatin) and amœboplasm (cytoplasm); thirdly, the Vegetable Kingdom, in which true

cells have entered upon an additional symbiosis with Cyanophyceæ, chromatophores or chlorophyll-corpuscles.

Interesting and suggestive as are the speculations of Mereschkowsky, they are nevertheless open to criticism from many points of view. I will not enter here into criticisms which I regard as beyond my competence. It is for botanists to pronounce upon the notion that Bacteria, Cyanophyceæ, and Fungi can be classified together as a group distinct from all other living beings; to decide whether the protoplasm of the Cyanophyceæ and Fungi can be regarded as consisting of mycoplasm alone, and not of a combination of nuclei and cytoplasm, such as is found in true cells and represents, according to Mereschkowsky, a symbiosis of mycoplasm and amœboplasm. I think I am right in saying that botanists are agreed in regarding Fungi as derived from green algæ, and as possessing nuclei similar to those of the higher plants. As a zoologist the point that strikes me most is the absence of any evidence that true Monera, organisms consisting of cytoplasm alone, exist or could ever have existed. Mereschkowsky supposes that when the Monera came into being they maintained their existence by feeding upon Bacteria. In order to digest Bacteria, however, the Monera must have been capable of producing ferments, and therefore did not acquire this power only as the result of symbiosis with Bacteria, unless it be assumed that the symbiosis came about at the instant that amœboplasm came into existence. There is, however, no evidence that cytoplasm by itself can generate ferments. All physiological experiments upon the digestion of Protozoa indicate that the cytoplasmic body, deprived of the nucleus, can not initiate the digestive process. Consequently the existence of purely cytoplasmic organisms would seem to be an impossibility.

For my part, I am unable to accept any theory of the evolution of the earliest forms of living beings which assumes the existence of forms of life composed entirely of cytoplasm without chromatin. All the results of modern

investigations into the structure, physiology and behavior of cells on the one hand, and of the various types of organisms grouped under the Protista, on the other hand—the combined results, that is to say, of cytology and protistology—appear to me to indicate that the chromatin-elements represent the primary and original living units or individuals, and that the cytoplasm represents a secondary product. I will summarize briefly the grounds that have led me to this conviction, and will attempt to justify the faith that I hold; but first I wish to discuss briefly certain preliminary considerations which seem to me of great importance in this connection.

It is common amongst biologists to speak of "living substance," this phrase being preceded by either the definite or the indefinite article—by either "the" or "a." If we pause to consider the meaning of the phrase, it is to be presumed that those who make use of it employ the term "substance" in the usual sense to denote a form of matter to which some specific chemical significance can be attached, which could conceivably be defined more or less strictly by a chemist, perhaps even reduced to a chemical formula of some type. But the addition of the adjective "living" negatives any such interpretation of the term "substance," since it is the fundamental and essential property of any living being that the material of which it is composed is in a state of continual molecular change and that its component substance or substances are inconstant in molecular constitution from moment to moment. When the body of a living organism has passed into a state of fixity of substance, it has ceased, temporarily or permanently, to behave as a living body; its fires are banked or extinguished. The phrase "living substance" savors, therefore, of a *contradictio in adjecto;* if it is "living" it can not be a "substance," and if it is a "substance" it can not be "living."

As a matter of fact, the biologist, when dealing with purely biological problems, knows nothing of a living substance or substances; he is confronted solely by living in-

dividuals, which constitute his primary conceptions, and the terms "life" and "living substance" are pure abstractions. Every living being presents itself to us as a sharply-limited individual, distinct from other individuals and constituting what may be termed briefly a microcosmic unit, inasmuch as it is a unity which is far from being uniform in substance or homogeneous in composition, but which, on the contrary, is characterized by being made up of an almost infinite multiplicity of heterogeneous and mutually interacting parts. We recognize further that these living individuals possess invariably specific characteristics; two given living individuals may be so much alike that we regard them as of the same kind or "species," or they may differ so sharply that we are forced to distinguish between them specifically. Living beings are as much characterized by this peculiarity of specific individuality as by any other property or faculty which can be stated to be an attribute of life in general, and this is true equally of the simplest or the most complex organisms; at least we know of no form of life, however simple or minute, in which the combined features of individuality and specificity are not exhibited to the fullest extent. A living organism may be so minute as to elude direct detection entirely by our senses, even when aided by all the resources furnished by modern science; such an organism will, nevertheless, exhibit specific properties or activities of an unmistakable kind, betraying its presence thereby with the utmost certainty. The organisms causing certain diseases, for example, are ultra-microscopic, that is to say, they have not been made visible as yet, and an exact description or definition can not be given of them at the present time; yet how strongly marked and easily distinguishable are the specific effects produced by the organisms causing, respectively, measles and small-pox, for instance, each, moreover, remaining strictly true and constant to its specific type of activity; the organism, whatever its nature may be, which causes measles can not

give rise to small-pox, nor *vice versa,* but each breeds as true to type as do lions and leopards.

The essential and distinctive characteristic of a living body of any kind whatsoever is that it exhibits while it lives permanence and continuity of individuality or personality, as manifested in specific behavior, combined with incessant change and lability of substance; and further, that in reproducing its kind, it transmits its specific characteristics, with, however, that tendency to variability which permits of progressive adaptation and gradual evolutionary change. It is the distinctively vital property of specific individuality combined with "stuff-change" (if I may be allowed to paraphrase a Teutonic idiom) which marks the dividing line between biochemistry and biology. The former science deals with substances which can be separated from living bodies, and for the chemist specific properties are associated with fixity of substance; but the material with which the biologist is occupied consists of innumerable living unit-individuals exhibiting specific characteristics without fixity of substance. There is no reason to suppose that the properties of a given chemical substance vary in the slightest degree in space or time; but variability and adaptability are characteristic features of all living beings. The biochemist renders inestimable services in elucidating the physico-chemical mechanisms of living organisms; but the problem of individuality and specific behavior, as manifested by living things, is beyond the scope of his science, at least at present. Such problems are essentially of distinctively vital nature and their treatment can not be brought satisfactorily into relation at the present time with the physico-chemical interactions of the substances composing the living body. It may be that this is but a temporary limitation of human knowledge prevailing in a certain historical epoch, and that in the future the chemist will be able to correlate the individuality of living beings with their chemico-physical properties, and so explain to us how living beings first came into existence; how, that is to say, a combination of chemical

substances, each owing its characteristic properties to a definite molecular composition, can produce a living individual in which specific peculiarities are associated with matter in a state of flux. But it is altogether outside the scope and aim of this address to discuss whether the boundary between biochemistry and biology can be bridged over, and if so, in what way. I merely wish to emphasize strongly that if a biologist wishes to deal with a purely biological problem, such as evolution or heredity, for example, in a concrete and objective manner, he must do so in terms of living specific individual units. It is for that reason that I shall speak, not of the chromatin-substance, but of chromatinic elements, particles or units, and I hope that I shall make clear the importance of this distinction.

To return now to our chromatin; I regard the chromatinic elements as being those constituents which are of primary importance in the life and evolution of living organisms mainly for the following reasons: the experimental evidence of the preponderating physiological *rôle* played by the nucleus in the life of the cell; the extraordinary individualization of the chromatin particles seen universally in living organisms, and manifested to a degree which raises the chromatinic units to the rank of living individuals exhibiting specific behavior, rather than that of mere substances responsible for certain chemico-physical reactions in the life of the organism; and last, but by no means least, the permanence and, if I may use the term, the immortality of the chromatinic particles in the life-cycle of organisms generally. I will now deal with these points in order; my arguments relate, in the first instance, to those organisms in which the presence of true cell-nuclei renders the identification of the chromatin-elements certain, as pointed out above, but if the arguments are valid in such cases they are almost certainly applicable also to those simpler types of organisms in which the identification of chromatin rests on a less secure foundation.

The results obtained by physiological experiments with

regard to the functions of the nuclear and cytoplasmic constituents of the cell are now well known and are cited in all the text books. It is not necessary, therefore, that I should discuss them in detail. I content myself with quoting a competent and impartial summary of the results obtained:

> A fragment of a cell deprived of its nucleus may live for a considerable time and manifest the power of coordinated movements without perceptible impairment. Such a mass of protoplasm is, however, devoid of the powers of assimilation, growth, and repair, and sooner or later dies. In other words, those functions that involve destructive metabolism may continue for a time in the absence of the nucleus; those that involve constructive metabolism cease with its removal. There is, therefore, strong reason to believe that the nucleus plays an essential part in the constructive metabolism of the cell, and through this is especially concerned with the formative processes involved in growth and development. For these and many other reasons . . . the nucleus is generally regarded as a controlling centre of cell-activity, and hence a primary factor in growth, development, and the transmission of specific qualities from cell to cell, and so from one generation to another.[20]

I may add here that the results of the study of life-cycles of Protozoa are entirely in harmony with this conception of the relative importance of nuclear—that is chromatinic—and cytoplasmic cell-constituents, since it is not infrequent that in certain phases of the life-cycle, especially in the microgamete-stages, the cytoplasm is reduced, apparently, to the vanishing point, and the body consists solely of chromatin, so far as can be made out. In not one single instance, however, has it been found as yet that any normal stage in the developmental cycle of organisms consists solely of cytoplasm without any particles of chromatin.

While on the subject of physiological experiment, there is one point to which I may refer. Experiments so far have been carried on with Protozoa possessing definite nuclei. It is very desirable that similar experiments should be conducted with forms possessing chromidia in addition to nuclei, in order to test the physiological capa-

[20] E. B. Wilson, "The Cell," second edition, 1911, pp. 30 and 31.

bilities of chromatin-particles not concentrated or organized. *Arcella* would appear to be a very suitable form for such investigations. This is a point to which my attention was drawn by my late friend Mr. C. H. Martin, who has lost his life in his country's service.

I have mentioned already in my introductory remarks that the only reliable test of chromatin is its behavior, and the whole of modern cytological investigation bears witness to the fact that the chromatinic particles exhibit the characteristic property of living things generally, namely, individualization combined with specific behavior. In every cell-generation in the bodies of ordinary animals and plants the chromatin-elements make their appearance in the form of a group of chromosomes, not only constant in number for each species, but often exhibiting such definite characteristics of size and form, that particular, individual chromosomes can be recognized and identified in each group throughout the whole life-cycle. Each chromosome is to be regarded as an aggregate composed of a series of minute chromatinic granules or chromioles, a point which I shall discuss further presently. Most striking examples of the individualization of chromosomes have been made known recently by Dobell and Jameson[21] in Protozoa. Thus in the Coccidian genus *Aggregata* six chromosomes appear at every cell-generation, each differing constantly in length if in the extended form, or in bulk if in the contracted form, so that each of the six chromosomes can be recognized and denoted by one of the letters *a* to *f* at each appearance, *a* being the longest and *f* the shortest.

(*To be continued.*)

[21] *Proc. Roy. Soc.* (B), Vol. 89. (In the press.)

THE EUGSTER GYNANDROMORPH BEES

PROFESSOR T. H. MORGAN

COLUMBIA UNIVERSITY

ABOUT fifty years ago von Siebold wrote his classic paper on "Zwitterbienen" in which he gave an account of anomalous bees that appeared in considerable numbers in a hive of a bee breeder, named Eugster, in Constance.[1] The particular interest that attached to the case was not only that a bee might be partly male and partly female, mixed in all manner of proportions, but that they were hybrid bees as well, the mother belonging to the race of Italian bees, while the father or fathers were German bees. Von Siebold did not state in his paper whether the male parts of the gynandromorph were like the father, or were hybrid, or were like the mother. In fact it was not until 1888 that the importance of such information was realized. In that year Boveri described a result that he had obtained with the eggs of the sea urchin, in which as a result of delayed fertilization (or of some irregularity in the penetration of the sperm into the egg) the sperm nucleus fused with one of the two nuclei resulting from the division of the egg nucleus. In consequence half of the nuclei were derived from the egg alone, while the other half of the nuclei arose from the union of the paternal and a maternal nucleus. If now, as other evidence seemed to show, one nucleus in the bee produces a male and two nuclei a female, such a partially fertilized egg should be male on one side and female on the other side of the body of the resulting individual. In this way, Boveri pointed out, the Eugster gynandromorphs might have arisen.

In 1905 I pointed out that the Eugster gynandromorphs might also be accounted for by means of another hypothesis. If two (or more) spermatozoa should enter the egg, one of them might unite with the egg nucleus while the

[1] Several earlier accounts of gynandromorph bees are extant (See "literature" list).

other might give rise to the nuclei of the rest of the embryo. On this hypothesis the combined nuclei would give rise to the female parts, while the single nucleus, here derived from the sperm, would give rise to the male parts. In support of such a view I pointed out that more than a single nucleus was known to enter the egg of the bee, and this condition has more recently been amply confirmed by Nachtsheim. I also pointed out, for the first time I believe, that a decision in favor of one or the other of these two hypotheses could be obtained if in these gynandromorph hybrids the nature of the male and of the female parts of the adult were known; for on Boveri's interpretation the male parts (derived from the single egg nucleus) should be maternal while on my view the male parts (derived from the single sperm nucleus) should be paternal. In both views the female parts of the gynandromorphs should be hybrid and therefore either intermediate in character or like the dominant strain.

Four years ago Professor Doflein looked through the collection at Munich, at the request of Boveri, to find out whether any of the Eugster bees were still preserved there, and luckily found a jar labelled "Apis Mellifica, Zwitterbienen" which turned out to be the bees that von Siebold had obtained. Owing to their long sojourn in alcohol the color was almost entirely gone and on the color depended the decision as to the difference between the two races that combined to produce the gynandromorphs. At first Boveri despaired of finding out from these alcoholic specimens whether the male parts were like the father or like the mother; but on cleaning the parts he found that he could still determine whether a part was more like the same part in one or in the other domesticated strain.

Briefly Boveri finds that the male parts of the gynandromorphs are maternal, while the female parts are paternal, which is the dominant character. This conclusion gives a decisive answer in favor of his hypothesis and sets my own aside for this case at least.

Boveri's evidence leaves no reasonable doubt as to the possibility of determining the nature of the character of

the gynandromorphs, yet the desirability of having it confirmed on living material may be still worth while, since, as Boveri points out in a postscript, von Engelhardt has recently (1914) described some hybrid gynandromorphs from fresh material which lead to the opposite conclusion from that to which Boveri has arrived. Von Engelhardt's bees arose from an Italian queen by a "domestic" drone. Until it is ascertained what variety was used as the domestic drone the value of the evidence is not entirely certain.

A student of Boveri's, Fr. Elsa Mehling, has made a very careful study of the Eugster gynandromorphs, paying attention to a number of characters. Her work adds many details of interest concerning the admixture of male and female parts, but does not, however, furnish much additional evidence concerning the origin of these parts. She arrives at the same conclusion as that reached by Boveri, viz., that the male parts are maternal.

In this connection it should be recalled that the long sought for evidence demonstrating that drones inherit the characters of their mother has at last been found by Newell. Working at an isolated station forty miles from Houston, Texas, he mated Italian and Carniolan races of bees. The Italians are distinctly yellow, while the Carniolans are more or less gray. The stocks used had been under observation for several generations and were known to be pure. When virgin Italian queens were mated to Carniolan drones the workers and queens (both of which come from fertilized eggs) are like the Italian yellow stock, which is, therefore, dominant as to color. The drones from this mating are also yellow, which is expected if they inherit from their mother, but the cross made this way is not decisive in regard to the inheritance of the drones, because the maternal color is here dominant. In the reciprocal cross the result is decisive. Thus when a Carniolan queen is mated to an Italian drone the workers and queens are yellow due to the dominant color of the father, but the drones are gray like the pure Carniolan drones. This result proves that the characters of the

drones come from the mother, which is in accord with Dzierzon's theory that the drones arise from unfertilized eggs. This is further established by the following evidence. The daughters (queens) that come from Italian queens by Carniolan drones give rise to two kinds of drones in equal numbers, viz., Italian and Carniolan, which is the expected result, since such daughters are hybrid and are expected to produce two kinds of eggs. Reciprocally also the daughters from Carniolan queens by Italian drones produce two kinds and only two kinds of drones in equal numbers. The result also shows that Mendel's law applies to the queen bee. Cuénot has recently recorded the appearance of some drones in hybrid hives that are intermediate or even like the father, but since the possible production of drones by hybrid workers was not excluded, at least so far as the published evidence goes, these sporadic cases can not be used to disprove the maternal inheritance of the drones.

Boveri has discussed certain cytological possibilities in relation to the gynandromorph bees that are of interest. His work, and that of Herbst on sea-urchin embryos, had shown that haploid nuclei have only half the volume of diploid nuclei. It might have been anticipated therefore that the nuclei (and cells) of the drone bee would be half the size of those of the queen or of the worker bee, but a study of the cells of drones by Oeninger had already shown that their nuclei are as large as are those of the workers which have the diploid number of chromosomes. It is not possible therefore to determine by microscopic study of nuclear size whether or not the male parts of gynandromorphs come from a single nucleus.

Boveri points out that, since the nucleus of the egg of the bee, if not fertilized, proceeds to divide, it is improbable that the division center is brought in by the sperm, as appears to be the case in so many other eggs. Nachtsheim's observations confirm, he believes, this interpretation in the bee; for, according to Nachtsheim, three to seven or more nuclei enter but only one of these fuses with the egg nucleus. The others move out into the egg,

their chromosomes are resolved, and a spindle develops. But these spindles lack centrioles at their poles. The mitotic figure that has reached this stage then proceeds to degenerate. The absence of the centrioles indicates, Boveri thinks, that the spermatozoa of the bee does not bring in a division center, hence this cell organ must be contributed by the egg, and in consequence we can now easily understand how facultative parthenogenesis is, so to speak, a normal phenomenon in this egg. Boveri does not point out however that Nachtsheim's figures show that the polar spindles of the bee's egg also lack centrioles, and yet mitotic division is accomplished. It seems highly questionable therefore whether much weight is to be attached to the absence of centrioles in the supernumerary sperm figures. The chief interest that attaches to Boveri's argument is his disclaimer that he intended his striking statement in regard to fertilization, namely, that the sperm furnishes the dynamic division center for development, to be taken as a universal dictum. The incitement of artificial division centers in such eggs as those of the sea urchin in which the sperm brings in the centriole (or causes its development in the immediate vicinity of the sperm nucleus) shows how little importance can be attached to the hypothesis of the genetic continuity of the centrosome. If in the case of the bee three or more sperm enter each egg all bees would be gynandromorphs should all the sperm develop. Obviously, some special condition must be assumed to be present if these sperms are to go forward and complete their development which they begin even under ordinary circumstances. Boveri himself must also invoke some special condition, such as retarded fertilization, in order that one of the entering sperm fuses with one of the products of the first division of the egg nucleus. It might equally well be postulated that delay in the fertilization and the consequent impetus to parthenogenesis might be favorable for the completion of the division of the supernumerary asters. In a word it is doubtful if Boveri's interpretation gains much from his cytological argument. If his observations on the dis-

tribution of color are well established this further argument is superfluous.

In 1906 Toyama described a gynandromorph that arose when two races of silkworm moths were crossed. From an analysis of the genetic evidence I pointed out that in this case the male parts of the gynandromorph must have been paternal and the hybrid parts maternal (dominant). If the same conditions prevail here as in the bee, viz., one nucleus producing a male and two producing a female,[2] the case is in harmony with my hypothesis and not with that of Boveri. But the evidence for my view is not as strong as that Boveri's is now for the bee; yet it may be true, nevertheless, that in both of these ways gynandromorphs may arise. A third mode of origin has been shown, from the genetic evidence, to apply to *Drosophila,* viz., dislocation during ontogeny of the two sex chromosomes. In fact we should expect that gynandromorphs would arise in insects whenever certain nuclei come to contain two sex chromosomes and others only one. The means by which this segregation takes place may differ under different conditions.

Goldschmidt has recently explained the remarkable gynandromorphs that he obtains in crosses between *Lymantria dispar* and *L. japonica* in still a different way, one that involves the relative potencies of the sex factors in the different races.

LITERATURE CITED

Boveri, Th. 1888. Über partielle Befruchtung. *Sitz.-Ber. d. Ges. f. Morph. u. Phys.,* Münch., IV.

Boveri, Th. 1915. Über die Entstehung der Eugsterschen Zwitterbienen. *Arch. f. Entw. Organismen,* XLI.

Cuénot, L. 1909. *Comp. Rend. Soc. Biol.,* LXVI.

Dönhoff. 1860*a*. Ein Bienenzwitter. *Bienenzeitung.*

[2] Whether one is justified in applying to the case of the moth the hypothesis for the bee may be seriously questioned because in the case of the moth the male is assumed to be the result of one sex chromosome (*z*) in conjunction with the *haploid* number of autosomes, while in the female moth one sex chromosome (*z*) and its mate (*w*) (which from Doncaster's evidence has no sex-determining influence) in conjunction with the *diploid* number of autosomes is assumed to stand for the female soma.

Dönhoff. 1860*b*. Beitr. z. Bienenkunde. I. Über Zwitterbienen. *Bienenzeitung.*

Goldschmidt, R. 1912. Erblichkeitsstudien an Schmetterlingen, I. *Zeit. f. ind. Abst. und Vererb.*, VII.

Hamet, H. 1861. Apiculture. *Revue et Magas. de Zool.*, XIII.

Laubender, B. 1801. Einige Bemerkungen über die von Herrn Schulmeister Lukas neu entdeckten Stacheldrohnen. *Ökonom.*, Hefte XVII.

Lefebure, A. 1835. Description d'un Argus Alexis hermaphrodite... *Ann. soc. entom. France*, IV.

Menzel, A. 1862*a*. Abnormität in der Bildung einer Biene. *Bienenzeitung.*

Menzel, A. 1862*b*. Über Zwitterbienen. *Bienenzeitung.*

Menzel, A. 1862*c*. Hymenopterologische Beobachtung I. Über die Geschlechtsverhältnisse der Biene im allgemeinen und über die Befruchtung der Königin, über Parthenogenesis und Zwitterbildung im besonderen. *Mitt. Schweiz. entom. Ges.*, Bd. I, Heft 2.

Menzel, A. 1864. Tod der Zwittermutter des Eugsterschen Stockes in Konstanz. *Bienenzeitung*, XX.

Morgan, T. H. 1905. An alternative interpretation of gynandromorphous insects. *Science*, XXI.

Morgan, T. H. 1907. The Cause of Gynandromorphism in Insects. *Am. Nat.*, XLI.

Morgan, T. H. 1909. Are the Drone Eggs of the Honey-Bee Fertilized? Am. Nat., XLIII.

Morgan, T. H. 1909. Hybridology and Gynandromorphism. Am. Nat., XLIII.

Nachtsheim, H. 1913. Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.). *Arch. f. Zellforsch.*, XI.

Newell, W. 1914. Inheritance in the Honey Bee. *Science*, XLI.

Oehninger, M. 1913. Über Kerngrössen bei Bienen. *Verh. d. phys.-med. Ges. Würzburg*, XLII.

Siebold, v.C. Th. 1864 Ueber Zwitterbienen. Zeit. f. wissensch. Zool. XIV.

Siebold, v.C. Th. 1866 Ersatz der abgestorbenen Zwittermutter des Eugsterchen Zwitterstockes in Constanz. Bienenzeit. Jahrg. 1866.

Smith, F. 1862. Imperfect Hermaphrodite of *Apis mell.* from Scotland. *Proc. Entom. Soc. London.*

Smith, F. 1871. Notes on Examples of Gynandromorphism in Aculeate Hymenoptera. *Trans. Entom. Soc. London.*

Toyama. 1906. On Some Silk-worm Crosses, with Special Reference to Mendel's Law of Heredity. *Bull. of the Coll. of Agr., Tokyo*, XII.

Wheeler, W. M. 1903. Some New Gynandromorph Ants. Bull. Am. Museum Nat. Hist. New York.

Wheeler, W. M. 1910. The Effects of Parasitic and other kinds of Castration in Insects. Jour. Exp. Zool. VII.

Wittenhagen. 1861. Über Bienencharakteristik und Bienenzwitter. *Bienenzeitung.*

SHORTER ARTICLES AND DISCUSSION

PINK-EYED WHITE MICE, CARRYING THE COLOR FACTOR

AMONG the many domesticated varieties of the house mouse (*Mus musculus*), two sorts with entirely white pelage are known, —the albino, and the black-eyed white. Numerous experiments have shown that the albino differs from colored varieties by the loss of a single factor, the color factor; for, in crosses with colored varieties, albinism acts as a recessive allelomorph. The genetic composition of the black-eyed white is less well known although several hypotheses have been suggested. Black-eyed whites possess the color factor as crosses with albinos have shown. They may be homozygous in the factor for dark eyes. A black-eyed white male produced 189 dark-eyed offspring in my experiments when mated to pink-eyed intense brown females. The offspring of this cross were heterozygous in dark eye (Dd). By mating them inter se, pink-eyed forms were obtained in the F_2 generation, some of which had a pure white coat. In other words, it is possible to recombine the factors producing the pure white pelage of the black-eyed whites with the pink-eyed condition. Such pink-eyed whites resemble true albinos in appearance, but not in zygotic constitution, for they still retain the color factor although they show no color. To avoid confusion in discussion, I shall refer to this synthesized form of albino as a pink-eyed white to distinguish it from the albino lacking the color factor. Predictions often compel subsequent retractions; however, I feel safe in predicting colored offspring from a cross between the pink-eyed white and the albino, although externally the mating resembles a cross between albinos which always breed true. Black-eyed white strains sometimes show a few colored hairs around the ears, between the eyes, and in front of the tail. The corresponding pink-eyed white forms may also show the same characteristic.

The white coat and pink eyes of the albino mouse are due to the loss of a single factor; but the white coat of the black-eyed white strains cannot be accounted for in such a simple manner. Little ('13) seemed inclined to the view that the black-eyed white mouse was a spotted individual in which the spotting was of the

recessive type, in contradistinction to spotting of the dominant type described by Miss Durham ('08). Through the kindness of Professor W. E. Castle, a black-eyed white male was received in the fall of 1914. With this male it was possible to produce other black-eyed whites. In such black-eyed whites as I have been able to test, both dominant and recessive spotting were present. Furthermore, the recessive spotting always occurred in double dose. Hence, black-eyed whites were supposed to have the zygotic formula PPss or Ppss, in which P stands for dominant spotting and p for its absence; and s represents the factor for recessive spotting which is allelomorphic to self (S). So far, I have been able to test sufficiently only two black-eyed white males, both of which were clearly of the formula Ppss. When mated to self-colored females they gave 231 offspring. Since these offspring showed much variability, they were graded in classes ranging from self (—9) to black-eyed white (+9) according to the amount of pigmentation which they showed. A distinct grouping around two modes was found as follows:

Class modes..	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9
Frequencies..	98	24	8	16	33	26	8	4	6	2	3	1	1	1	0	0	0	0	0

About one-half of the F_1 offspring was grouped around the lower mode (126), and the other half (105) grouped around the upper mode, if we assume the class —7, as the dividing class. Very few individuals were found in the "doubtful class." Expressing the cross of black-eyed white with self-colored in Mendelian terms, it would be:

Ppss × ppSS = P_1 zygotes
Ps + ps = gametes of black-eyed white P_1
pS + pS = gametes of self-colored P_1
PpSs + ppSs = F_1 zygotes
Spotted + Self

The results conformed to this expectation. The individuals grouped around the lower mode were self-colored or very nearly so, as one would expect of individuals heterozygous in self and recessive spotting, for self is dominant or very nearly dominant to recessive spotting. Their formula was ppSs. Subsequent experiments corroborated this, for they produced self and recessive spotted in Mendelian ratios, when mated inter se or to recessive spotted individuals. They never gave black-eyed whites in

such matings. Those offspring grouped around the upper mode were spotted, and had a formula PpSs. When mated inter se or back to recessive spotted, they gave, besides spotted and selfs, black-eyed whites; apparently because the combination Ppss could again be formed. The dominant spotting factor, P, evidently acts more vigorously upon recessive spotting than upon self. It can not restrict the more extended pigmentation of a self coat completely. Hence, half of the F_1 individuals (those with the formula PpSs) were spotted, or, to describe them more accurately, spotted with frequent and varying amounts of silvering. The dominant spotting factor, P, can, however, restrict the limited pigmentation of a recessive spotted coat completely or almost completely. Hence animals with the formula Ppss were black-eyed whites.

The origin of our new pink-eyed white forms, which resemble albinos so closely as to be indistinguishable from them, is evidently due to the substitution of the pink-eye factor for dark-eye in black-eyed whites, and not due to the loss of the color factor C. In our cultures, the black-eyed whites have the formula PpssDDCC and the corresponding pink-eyed whites had the formula PpssddCC where D and d represent dark eye and pink eye respectively, and C represents the color factor. We have also produced black-eyed white forms heterozygous in dark eye, PpssDd. Black and brown are likewise interchangeable in the dark-eyed whites, for black-eyed whites, heterozygous in black, have been produced. I see no reason why brown-eyed whites can not be produced in the usual Mendelian fashion by mating black-eyed whites to browns, and recovering the white pelage with brown eyes in the F_2 generation. Mating the spotted F_1 offspring inter se should give, among others, individuals with the formula PpssbbCC. These would be brown-eyed whites,—white because of the combined action of P and s, and brown simply because they lack the differential factor B which changes brown into black.

The occurrence of pink-eyed whites which resemble albinos may have some bearing on an anomalous case cited by Bateson ('04) as follows: "the production of colored animals by albinos, is not, so far as I know, illustrated by a single case, with the following exception. In the later editions of "Fancy Mice" (Upcott Gill), Dr. Carter Blake, formerly secretary of the Anthropological Institute commenting on the statement that albino mice of whatever

parentage produce nothing but albinos, writes that a pair of albinos produced some brown-and-white, some plum, some grey, and some albinos. If this result occurred under all precautions, it stands alone.'' Allen ('04) attempted to account for this case by postulating an error in recording the true sire, or that the animals used were not true albinos but black-eyed whites. That two individuals having white coats and pink eyes can give colored young is perfectly possible. The pink-eyed whites in my cultures have a white pelage because of the combined effect of the dominant and recessive spotting, while their pink eye is due to the loss of the dark-eye factor. They still retain the color factor, although they show no color. They may be called albinos, if we define an albino as any pink-eyed white individual; but they should be carefully distinguished from that type of albinism which is due to the loss of the color factor. If we mate these two different types of albinos together, we should obtain colored young. The cross may be expressed in symbols:

PpssddCC × ppSSDDee ..... P. zygotes
PsdC + psdC ..... gametes of pink-eyed white
pSDe + pSDe ..... gametes of albino
PpSsDdCe + ppSsDdCe ..... F_1 zygotes
Spotted + Selfs

It is interesting to note that the exceptional case, quoted by Bateson, mentions the occurrence of spotted and selfs in the cross of two albinos. In plants, as in animals, similar somatic characters do not necessarily indicate similar germinal constitution.

Our assumption of the interaction of a dominant and recessive spotting factor to account for the white pelage of pink-eyed and black-eyed whites is strengthened by the valuable paper of Little ('15). Little has adopted a similar hypothesis for black-eyed whites in his paper just published, and quite different from the hypothesis of his earlier paper ('13). It should be stated that Little's experiments furnish even a larger amount of data from the more convincing type of matings than has been possible in our own cultures as yet.

J. A. DETLEFSEN.

REFERENCES

Allen, G. M. 1904. Proc. Am. Acad. Arts and Sci., vol. 40, pp. 61–163.
Bateson, W 1903. Proc. Zool. Soc. London, vol. 2, pp. 71–98.
Durham, F. M. 1908. Royal Soc., Rep. Evol. Comm., No. 4, pp. 41–53.
Little, C. C. 1913. Carnegie Inst. Wash., Pub. 179, pp. 11–102.
Little, C. C. 1915. Am. Nat., vol. 49, pp. 727–740.

PARTHENOGENESIS AND SEXUAL REPRODUCTION IN ROTIFERS. EXPERIMENTAL RESEARCH UPON BRACHIONUS PALA[1]

IN a recent number of *Bios* Miss Lina Moro has presented some interesting and suggestive results from experiments upon the rotifer, *Brachionus pala*. She has subjected the parthenogenetic females to various chemicals, to changes in nutrition, and to changes in temperature.

In using $FeCl_3$ solutions she has been able to produce male-producing females in small numbers while in control experiments in which no $FeCl_3$ was used no male-producing females were produced. Many dilutions of $FeCl_3$ were used but $M/12{,}000$ seemed to be the optimum dilution. This was added to the culture water of hay infusion in which the rotifers were living. Although the number of the experiments were rather small and the percentage of male-producing females obtained was not higher than 12 per cent., nevertheless they indicate the possibility of a specific chemical being able to induce the production of male-producing females.

Not only did $FeCl_3$ cause male-producing females to appear but it also caused the mothers to form the eggs much faster in their bodies and to extrude them to the outside much faster than those in the controls. Usually while a female in the control was producing one egg a female in the $FeCl_3$ would produce four eggs. This rapid formation and production of eggs after it was once started continued through many subsequent generations during the three months in which the experiments were carried on. It might be considered that this new characteristic induced by a chemical was a case of the formation of a new character which, after it was once formed, was inherited by the descendants.

It was also determined that the influence of the $FeCl_3$ acted upon the egg while it was yet inside the mother and caused it to develop into a male-producing female. After the egg was laid its development could not be altered from a female-producing female to a male-producing female by the use of $FeCl_3$.

A dilution of $HgCl_2$ ($M/1{,}200{,}000{,}000$) was also effective in causing male-producing females to appear but a smaller number of offspring were produced than in the $FeCl_3$. The percentage

[1] "Partenogenesi e Anfigonia nei Rotiferi. Recerche sperimentali sul *Brachionus pala*," by Lina Moro, *Bios*, Vol. 2, Fasc. 3, pp. 219–264, 1915.

of male-producing females produced was about 18 per cent. It also caused an increase in the number and the rate of production of the eggs by each female as was the case in the $FeCl_3$ experiments. KCl ($M/12{,}000$) in the very few experiments recorded caused about 16 per cent. of male-producing females to appear and $CaCl_2$ ($M/12{,}000$) caused about 33 per cent. of male-producing females to appear. In the controls for these experiments no male-producing females appeared. In all of these chemical experiments each mother after being transferred from the control to the culture media containing the various chemicals produced a family of several daughters but in each family there was never more than *one* male-producing daughter. In the $FeCl_3$ solutions each mother produced many daughters and as only one of them in each family was a male-producer the percentage of male-producing females was necessarily lower whereas in the $HgCl_2$, KCl, $CaCl_2$, solutions each mother produced fewer daughters than in the $FeCl_3$ solutions, and as only one of these in each family was a male-producer, the percentage of male-producing females was consequently higher. Various dilutions were used of $AlCl_3$, KCN, NaCl, Na_2HAsO_4, HCl, and NaOH but none of them caused male-producing females to appear.

In the nutrition experiments it was found that a constant diet at a uniform temperature of 15° C.–17° C. or 25° C.–27° C. produced only female-producing females but in some experiments in which an abundance of food was used for a time and then was followed by a period of scanty food or semi-starvation many male-producing females appeared, especially at the lower temperature.

In some of the experiments a temperature of 15° C.–17° C. produced all female-producing females but when the mothers were put at a temperature of 25° C.–27° C. or at 31° C. as high as 50 per cent. of the daughters were male-producers. When these same mothers were transferred back to 15° C.–17° C. they again produced only female-producing daughters. In a few experiments at a constant temperature of 25° C.–27° C. only female-producing females were produced but when the mothers were put at a lower temperature they produced many male-producing daughters. The general conclusion drawn is that whenever the general cultural conditions are constant and uniform, whether they refer to nutrition or to temperature, only female-producing females are produced but when the cultural conditions are sud-

denly changed by the disappearance of an abundant diet or by the rise or fall in the temperature male-producing females are produced at once. In a few experiments very young females (1–7 hours after hatching) were put from a high temperature to a temperature of 9° C.–11° C. and many of them developed into male-producers but whether this was due to the temperature or to some other factor was not known.

Another fact of considerable interest was verified. It concerned the nature of the male-producing females and the sexual females (the females which produce fertilized eggs). It has been observed by several investigators that if the small male eggs of a male-producing female are fertilized, in a species of *Asplancha* and *Hydatina senta,* they develop into the winter or resting eggs. This was found to be true also in *Brachionus pala.*

In all the families of daughters from the various mothers it was found that the male-producing daughters were among the earliest ones produced of each family. This was observed in the families of *Hydatina senta* by an earlier worker but later it was found to be due entirely to the method of feeding.

Although, as stated previously, the observations recorded in this paper are from a rather small number of individuals and ought to be expanded and verified, nevertheless, they show that in this rotifer the production of female-producing or male-producing females can be regulated by the environment and thus the results are in a general accord with the observations obtained by several workers with the rotifers, *Asplancha,* and *Hydatina senta.*

D. D. WHITNEY

NOTES AND LITERATURE

AN OUTLINE OF CURRENT PROGRESS IN THE THEORY OF CORRELATION AND CONTINGENCY

WORKERS in the physical sciences realized long ago that certain progress depended upon the precision of their instruments of measurement and the adequacy of their methods of mathematical description and analysis. Biologists, here and there, are beginning to see the importance of the analytical as well as of the observational tools. Among the analytical formulæ none are of greater usefulness than those for measuring interdependence. It may not be out of place, therefore, to sketch in simple terms for the benefit of those who are interested in the methods only as a means to an end, the progress which is being made in the perfection of these instruments of research.

The term *current* as used in these paragraphs is made more comprehensive than is conventional; some of the citations are four or even more years old. The elasticity of the term is justified in dealing with the literature of a field in which progress is particularly difficult and in which actual contributions are incorporated but slowly into the working technique of the biologist. Indeed, biologists as a class still think of correlation as synonymous with the classical product-moment method. How erroneous this impression is will appear in the following pages.

The purpose of this review is therefore to indicate in nonmathematical terms easily comprehensible to biological readers the lines of advances in the theory of the measurement of interdependence in order that they may the more easily select for dealing with their actual data, formulæ of the existence of which they might otherwise be unaware.

The progress which we have to consider has been along four different lines:

(*a*) In the simplification of methods of computation in the case of familiar formulæ. (*b*) In the development of entirely new formulæ applicable to data of particular sorts. (*c*) In the determination of the corrections to be applied for grouping into "broad categories." (*d*) In partial correlation, multiple correlation, and the correlation of indices and increments.

In this review we shall limit ourselves strictly to an outline of progress which has been made in the theory of the measurement of the interrelationship of two variates, leaving for consideration at a later time the far more complex subjects of correction for grouping, partial and multiple correlation, variate difference correlation and some other topics.

The detailed advances may be most easily understood by considering the kinds of data with which one has to deal in determining the degree of interdependence, association or correlation (to use these terms in a broad sense) between two variates.

An arrangement of the literature according to a key similar to that familiar in taxonomic works will perhaps be of service to the biologist who desires to locate at once the literature pertinent to the particular kind of data with which he has to deal.

Suppose first of all that the two characters are both suitable for measurement (or counting) on a quantitative scale and that for both the measurements form several classes. The choice of methods for measuring the correlation between them will then depend upon whether the average values of the y character associated with serially arranged values of the x character lie in sensibly a straight line or whether they can best be represented by some more complex curve. Linearity of regression, as it is technically called, has therefore a two-fold significance. (*a*) Biologically, it shows that an associated character changes at a *uniform* rate (however slight this rate may be) with the variation of a selected character. (*b*) Statistically, it justifies the application of the familiar product-moment method of determining the correlation coefficient.

Both Characters Measurable on a Quantitative Scale, Regression Linear.—So satisfactory has the product-moment method proved for data in which both characters are measurable and regression is sensibly linear, that no fundamental advance has been made for several years. Boas's[1] first formula is, as pointed out by Pearson,[2] merely another form of the difference method, which has been in use for many years.

Several modifications of a purely technical nature which facilitate calculation or are useful in special cases have been pub-

[1] Boas, F., "Determination of the Coefficient of Correlation," *Science*, N. S., *29:* 823–824. 1909.

[2] Pearson, K., "Determination of the Coefficient of Correlation," *Science*, N. S., *30:* 23–25, 1909.

lished. Pearson[3] has given a new approximate difference method which is serviceable in special cases only. Harris[4] has suggested a novel difference method for exact work with tables. An alternative method of calculating rough moments and product moments, given by Elderton,[5] seems to have attracted little attention, although it has certain advantages for use in adding-machine computations. A product moment method which possesses marked advantages for use with machines which allow of simultaneous multiplication and summation, and which obtains incidentally the data necessary for testing linearity of regression or computing the correlation ratio, η, is now available.[6] In the special cases in which the two characters to be centered in the correlation table are not differentiated, *e. g.*, stature of pairs of brothers, length of *Paramecium*, etc., the tables are ordinarily rendered symmetrical by using each individual once as the x and once as the y member of the pair. This may be done by actually forming the symmetrical table, or by using the simple formula proposed by Jennings.[7] If, as is frequently the case, more than a single pair of individuals are associated, the labor of forming tables becomes very great. Each individual of a family, each organ of an individual, or each individual measured from a particular environment, must then be entered in the table in combination with every other one. Since the number of combinations in each class is $n(n-1)$ and the number of classes must be at least moderately large, the total number of combinations is very great. Thus the data for number of nipples in swine recently published by Parker and Bullard[8] require a table of 34,884 combinations to determine the fraternal correlation for number of nipples. In the case of the *Hydra* data analyzed by Lashley,[9] tables with from one to nearly two hundred thousand

[3] Pearson, K., "On Further Methods of Determining Correlation," Drapers' Company Research Mem., Biom. Ser., IV, Dulan and Co., 1907.

[4] Harris, J. Arthur, "A Short Method of Calculating the Coefficient of Correlation in the Case of Integral Variates," *Biometrika, 7:* 214–218, 1909.

[5] Elderton, W. P., "An Alternative Method of Calculating the Rough Moments from the Actual Statistics," *Biometrika, 4:* 374–378, 1905. Also in his "Frequency Curves and Correlation."

[6] Harris, J. Arthur, "The Arithmetic of the Product Moment Method of Calculating the Coefficient of Correlation," AMER. NAT., *44:* 693–699, 1910.

[7] Jennings, H. S., "Computing Correlation in Cases Where Symmetrical Tables are Commonly Used," AMER. NAT., *45:* 123–128, 1911.

[8] Parker, G. H., and C. Bullard, *Proc. Amer. Acad. Arts and Science, 49:* 399–426, 1913.

[9] Lashley, K. S., *Jour. Exp. Zool., 19:* 210, 1915.

combinations are given. Methods for the rapid formation of symmetrical tables from which either correlation or contingency coefficients may be calculated[10] and for the formation of condensed tables from which correlation coefficients[11] only may be deduced greatly reduce the necessary labor in such cases. For the testing of linearity of regression in the case of these intra-class and inter-class correlations, tables are essential. The use of such coefficients would, however, be greatly facilitated if calculation could be carried out directly from moments computed from the classes themselves. Harris[12] has given an exhaustive series of formulæ by which this can be accomplished, with examples showing the wide applicability of such coefficients. For example, these formulæ fulfil more adequately the purpose of Boas's second formula (*loc. cit.*).

These intra-class correlation formulæ have been thrown into a form suitable for measuring substratum heterogeneity in experimental cultures.[13]

If the x and y character of a pair are differentiated, spurious values of the correlation coefficient must result from the rendering symmetrical of the correlation surface. Pearson many years ago recognized the difficulty in dealing with groups in which there is orderly differentiation due, for example, to growth.[14] Attention has recently been directed[15] to difficulties arising when differentiation within the class may exist, but it may be difficult or impossible to arrange the individuals by any character outside of themselves to obtain the constants necessary for determining the true correlation from the spurious values deduced

[10] Harris, J. Arthur, "On the Formation of Correlation and Contingency Tables when the Number of Combinations is Large," AMER. NAT., *45:* 566–571, 1911.

[11] Harris, J. Arthur, "The Formation of Condensed Correlation Tables when the Number of Combinations is Large," AMER. NAT., *46:* 477–486, 1912.

[12] Harris, J. Arthur, "On the Calculation of Intra-class and Inter-class Coefficients of Correlation from Class Moments when the Number of Possible Combinations is Large," *Biometrika, 9:* 446–472, 1913.

[13] Harris, J. Arthur, "On a Criterion of Substratum Homogeneity or Heterogeneity in Field Experiments," AMER. NAT., *49:* 430–454, 1915.

[14] Pearson, K., "On Homotyposis in Homologous but Differentiated Organs," *Proc. Roy. Soc. Lond., 71:* 288–313, 1903.

[15] Harris, J. Arthur, "On Spurious Values of Intra-Class Correlation Coefficients Arising from Disorderly Differentiation within the Classes," *Biometrika, 10:* 412–416, 1914.

from the tables. Whether the methods used in such cases by Harris[16] will prove the best available remains to be seen.

Considerable attention has recently been given to the probable error of the correlation coefficient.

If the number of observations upon which r is based is large and if it does not approach too closely either of its limiting values of $+1$ or -1, the use of the formula of Pearson and Filon,

$$E_r = .6745\frac{1-r^2}{\sqrt{n}},$$

readily evaluated by the use of the tables of $1-r^2$ given by Soper[17] used in connection with the x_1, of Miss Gibson's Tables[18] or approximated by the Abac of Heron,[19] is quite legitimate. But when either of these conditions is not realized the value of r found from a single sample will probably not be the true correlation for the population under consideration.

Chemists, agriculturists, physiologists and many others often must necessarily reason from a relatively small number of observations. It is therefore of very real importance that some valid measure of the statistical trustworthiness of such coefficients be known. Some of the problems concerning the probable error of r when it approaches its numerical limits or when the number of cases upon which it is based is small are discussed mathematically by Soper[20] as they have been attacked experimentally by "Student."[21] Further contributions to the subject are those of Fisher[22] and of Pearson,[23] who summarizes the series of studies and gives a table to facilitate the interpretation of correlation coefficients based on small samples. He says:

[16] Harris, J. Arthur, "On the Significance of Variety Tests," *Science*, N. S., *36:* 318–320, 1912, and *Biometrika, l. c.*

[17] Soper, H. E., In "Tables for Statisticians and Biometricians."

[18] *Biometrika, 4:* 385–392, 1906. Also in Pearson's Tables.

[19] Heron, D., "An Abac for Determining the Probable Errors of Correlation Coefficients," *Biometrika, 7:* 411, 1910. Also in Pearson's Tables.

[20] Soper, H. E., "On the Probable Error of a Correlation Coefficient to a Second Approximation," *Biometrika, 9:* 91–115, 1913.

[21] "Student," "Probable Error of a Correlation Coefficient," *Biometrika, 6:* 302–310, 1908.

[22] Fisher, R. A., "Frequency Distribution of the Values of the Correlation Coefficient in Samples from an Indefinitely Large Population," *Biometrika, 10:* 507–521, 1915.

[23] Pearson, K., "On the Distribution of Small Samples"; Appendix I to papers by "Student" and R. A. Fisher, *Biometrika, 10:* 522–529, 1915.

We think it must be concluded that for samples of 50 the usual theory of the probable error of the standard deviation holds satisfactorily, and that to apply it for the case of $n = 25$ would not lead to any error which would be of importance in the majority of statistical problems.

The original papers should be read by those who are dealing with coefficients lying near the limits of the range of correlation, or who must work with small samples. Those who can by extra labor obtain larger series of data should do so, for no knowledge of the theory of the probable error can ever take the place of widened series of data, although it may be essential to the interpretation of constants based of necessity on a limited number of observations.

Both Variates Measurable on a Quantitative Scale; Regression Non-Linear.—For cases in which the rate of change in the y character can not be described by a straight line, the proper measure of interdependence is Pearson's[24] correlation ratio, η. The value of the correlation ratio is two-fold. (*a*) It furnishes a measure of the interdependence of two variates in cases in which the use of the correlation coefficient is not fully justified. (*b*) It affords a means of testing, by the use of Blakeman's criterion,[25] for linearity of regression. Thus in deciding between the correlation coefficient and the correlation ratio, the calculation of each of the constants may, in critical cases, be necessary.

A further test of the goodness of fit of regression curves has also been given by Slutsky.[26] This method, which involves the well-known χ^2 of Pearson's test for goodness of fit, should have wide usefulness. An illustration of its application has recently been given by Pearl.[27]

One Variate Describable in Multiple Categories, the other Measurable on a Quantitative Scale.—Such cases are occasionally met with in many fields of work. For example, one may desire to know in fractions of a scale ranging from 0 to 1 the relationship between any describable but not measurable environmental

[24] Pearson, K., "On the General Theory of Skew Correlation and Non-Linear Regression," Drapers' Co. Res. Mem., Biom. Ser., II, Dulan and Co., 1905.

[25] Blakeman, J., "On Tests for Linearity of Regression in Frequency Distributions," *Biometrika, 4:* 332–350, 1905.

[26] Slutsky, E., "On the Criterion of the Goodness of Fit of Regression Lines and on the Best Method of Fitting them to the Data," *Jour. Roy. Stat. Soc., 77:* 78–84, 1914.

[27] Pearl, R., "An Important Contribution to Statistical Theory," AMER. NAT., *48:* 505–507, 1914.

factor and any measurable characteristic of the organisms subjected to its influence. Or in testing the assertions of such writers on criminology as Lombroso and Havelock Ellis against the results of actual measurements of criminals, one may find it desirable to correlate between the kind of crime and any cephalic measurement.

For the analysis of such data the correlation ratio may be of great service.

One Character Alternative, the other Measurable on a Quantitative Scale.—Suppose now that one of the correlation ratio tables of the kind discussed in the foregoing paragraph were reduced, as far as the qualitatively appreciable but not measurable character is concerned, to two classes only, while the measured variate remained as before. Such tables actually occur in practise with great frequency. For example, one may wish to correlate between the form of a dimorphic crustacean and physical measurements. Or it may be desirable to ascertain the correlation between type (tubular or ligulate) of a composite flower and the number of divisions in the corolla. Or one may wish to measure the relationship between type and time required for germination in the seeds of a dimorphic plant species. Or a series of individuals may be classified by the social worker or prison warden as alcoholic and non-alcoholic and the investigator desires to correlate between alcoholism (which is really a graduated character, although classified in the available records into the two alternative classes only) and any physical measurement or the extent of criminality as measured by number of convictions or months spent in prison.

In this reduced form the data can no longer be treated by the correlation ratio method, but must be attached by a recent formula due to Pearson,[28] and known as the Bi-serial correlation coefficient.

Soper[29] has continued his work on the probable error by determining the standard deviation of constants calculated by this formula.

Both Characters Classified in Multiple Categories.—If instead

[28] Pearson, K., "On a New Method of Determining Correlation Between a Measured Character *A*, and a Character *B* of Which Only the Percentage of Cases Wherein *B* Exceeds (or Falls Short of) a Given Intensity is Recorded for Each Grade of *A*," *Biometrika, 7:* 96–105, 1909.

[29] Soper, H. E., "On the Probable Error of the Bi-serial Expression for the Correlation Coefficient," *Biometrika, 10:* 384–390, 1914.

of both characters being measurable on a quantitative scale, or one character recorded in a number of categories and the other measurable on a quantitative scale, *both* characters are not quantitatively measurable, but describable in a number of classes only, neither the correlation coefficient nor the correlation ratio can be used. In such cases, which in practical work are very frequent, Pearson's contingency methods[30] must be used. These have been too long in use to require discussion or illustration here. Certain corrections to be applied will be considered at another time.

The probable error of the contingency coefficient presents considerable difficulty. Those who have to deal with it should consult papers by Blakeman and Pearson[31] and by Pearson.[32]

One Variate Classified in Alternative, the Other in Multiple Categories.—Consider a contingency table reduced to a two-fold grouping for one of the characters, but retaining the multiple division for the other. Such a table is comparable with the condensation of the correlation ratio table discussed above. It must be analyzed by a special method.[33]

The formula has not as yet had extensive practical application. It has been used to determine the relationship between alcoholism as an alternative character and type of crime classed in multiple categories, and between alcoholism in the parent and health of the children. It may prove especially valuable in dealing with the interrelationship of various teratological conditions in morphological work.

Both Characters Classified in Alternative Categories Only.—As the extreme case we may think of a contingency table reduced to a two-fold grouping for each of the characters. This is then the four-fold table for alternative characters, *i. e.*, (A) and (not $-A$), (B) and (not $-B$).

In the past, two methods have been chiefly employed for obtaining constants from such tables, Pearson's four-fold correlation coefficient and Yule's coefficient of association.

[30] Pearson, K., "On the Theory of Contingency and its Relation to Association and Normal Correlation," Drapers' Co. Res. Mem., Biom. Ser., I. Dulan & Co., 1904.

[31] Blakeman, John, and K. Pearson, "On the Probable Error of Mean Square Contingency," *Biometrika, 5:* 191–197, 1906.

[32] Pearson, K., "On the Probable Error of a Coefficient of Mean Square Contingency," *Biometrika, 10:* 570–573, 1915.

[33] Pearson, K., "On a New Method of Determining Correlation when One Variable is Given in Alternative and the Other in Multiple Categories," *Biometrika, 7:* 248–257, 1909.

For several years critical workers have realized that very little reliance is to be placed upon Yule's very simple coefficient of association. This coefficient and another measure of correlation "the theoretical value of r" proposed in his "Introduction to the Theory of Statistics" have been discussed by Heron.[34] Pearson and Heron[35] and Pearson[36] have gone into these methods and others proposed by Yule[37] in a masterly way. To discuss this memoir alone would require far more than the space available for this general index of the correlation methods. Their treatment can leave no doubt—if any existed in the minds of those who have tried to use these formulæ in serious statistical work—that except in very special cases all these association and colligation formulæ are likely to work harm rather than to be of service in the hands of the biologist.

This demonstration of the untrustworthiness of the various substitutes for the correlation coefficient practically throws us back upon the old four-fold method of Pearson, and upon another novel method to be discussed in a moment. The difficulty of computation has been one of the greatest obstacles in the way of the more general application of this method and has frequently resulted in the substitution of the less reliable coefficient of association. The necessary labor of calculation has been much reduced by two series of tables by Everitt.[38]

The determination of the probable error of the coefficient of correlation calculated from the four-fold grouping has always been excessively laborious. While four-fold correlations have been calculated in hundreds of cases, the determination of the probable error has been made for less than a hundred of the coefficients. Pearson[39] has now given tables to facilitate the cal-

[34] Heron, D., "The Danger of Certain Formulæ Suggested as Substitutes for the Correlation Coefficient," *Biometrika, 8:* 109–122, 1911.

[35] Pearson, K., and D. Heron, "On Theories of Association," *Biometrika, 9:* 159–315, 1913.

[36] Pearson, K., "Note on the Surface of Constant Association," *Biometrika, 9:* 534–537, 1913.

[37] Yule, G. U., "On the Methods of Measuring Association between Two Variates," *Jour. Roy. Stat. Soc., 75:* 579–641, 1912.

[38] Everitt, P. F., "Tables of the Tetrachoric Functions for Four-fold Correlation Tables," *Biometrika, 7:* 437–451, 1909; "Supplementary Tables for Finding the Correlation Coefficient from Tetrachoric Groupings," *Biometrika, 8:* 385–395, 1912. Also in "Tables for Statisticians and Biometricians."

[39] Pearson, K., "On the Probable Error of a Coefficient of Correlation as

culation of approximate probable errors which are sufficiently exact for all practical purposes.

Finally, the most important recent development in the theory of correlation is probably Pearson's novel method of dealing with variates classed in alternate categories only.[40]

The fundamental conception of this method is exceedingly simple. Given the table,

	A_1	A_2	Totals
B_1	a	b	$a+b$
B_2	c	d	$c+d$
Totals	$a+c$	$b+d$	N

where the large letters represent any alternative (*e. g.*, Mendelian) characteristic of an individual, and the small letters denote the frequency of occurrence of the several possible combinations, it is clear that

$$\frac{a+c}{N}, \frac{b+d}{N}, \frac{a+b}{N}, \frac{c+d}{N}$$

give the independent probabilities of the two pairs of characteristics. The four pertinent products of these ratios give the chances on the assumption of the independence of the two characters A and B, of the four possible combinations. Then if there be no correlation, within the limits of the errors of random sampling

$$a - N\left(\frac{a+c}{N} \times \frac{a+b}{N}\right) = 0,$$

and so on. The squares of the four differences between the observed frequencies, a, b, c, d, and those which would be expected if the two characters were really independent, gives the familiar χ^2 of Pearson's test for goodness of fit. The significance of this test may be determined from Palin Elderton's tables,[41] and this is, in the case in hand, a measure of correlation. It has been

Found from a Four-fold Table," *Biometrika, 9:* 22–27, 1913. Also in Tables for Statisticians and Biometricians.

[40] Pearson, K., "On a Novel Method of Regarding the Association of Two Variates Classed Solely in Alternate Categories," Drapers' Co. Res. Mem., Biom. Ser., VII, Dulan and Co., 1912.

[41] Elderton, W. P., "Tables for Testing the Goodness of Fit of Theory to Observation," *Biometrika, 1:* 155–163, 1901. Also reprinted in Pearson's volume of tables.

used as such during the past several years by some of us in practical problems in which we found it impossible to place reliance upon Yule's coefficients and did not feel warranted, because of underlying assumptions, in depending solely upon the classical four-fold method. But it is a measure given in terms utterly incomprehensible to the ordinary mind, which is quite incapable of thinking in millions or in multiples of millions.

What Pearson has done with such brilliancy is to furnish a means in mathematical theory and working tables of passing from the incomprehensible scale of pure probability to the familiar and usable and widely comparable scale of correlation.

As yet it is too soon to be able to state the results of extensive practical application of the new coefficients, but they should have wide usefulness.

Both Characters Classified by Rank in Series.—In some cases, neither measurements nor classification of the individuals dealt with in categories are given in the data, but merely their position or rank in the series.

Rank may be numerically expressed, and the suggestion has been made that the correlation of grades or ranks is a quite legitimate measure of interdependence in such cases. Pearson[42] has, however, pointed out the very real difficulties encountered in such work. Those who are tempted to use these methods should acquaint themselves with the dangers as pointed out in this memoir.

One Variate Given by Rank in Series, the Other Measured on a Quantitative Scale.—Such cases are not likely to occur with great frequency in biological work. Possible instances are those in which one wishes to correlate between position in an intensity of pigmentation series and size or fertility—both quantitatively measurable characters. The formulæ have been given by Pearson.[43]

One Variate Given by Multiple or Broad Categories, the Other by Rank in Series.—Practical applications in biology should be rare. For formulæ see the paper by Pearson just cited.

From the foregoing outline it must be clear that of recent years the conception of correlation has been greatly extended and the possibilities of the practical usefulness of correlation methods

[42] Pearson, K., "On Further Methods of Determining Correlation," Draper's Co. Res. Mem., Biom. Ser., IV, Dulan and Co., 1907.

[43] Pearson, K., "On an Extension of the Method of Correlation by Grades or Ranks," *Biometrika, 10:* 416–418, 1914.

vastly increased by the deduction of formulæ suitable for dealing with data of the most diverse sorts.

The most valuable feature of a summary such as the present may possibly not lie in the fact that it exhibits to biologists the wide array of statistical tools which are now available for dealing with the most diverse sorts of data which they may collect, and shows where directions for their use may be found, but in the suggested warning that the hasty application of the first learned or the most easily calculated formula may lead to constants of little value. Most biologists can use a scalpel or a beaker with great success, but many at least would hesitate to try to handle without special training all the instruments which are to be seen in the surgeon's case or to use all the glassware on the organic chemist's shelves. Each kind of tools require their special training. Notwithstanding popular conceptions to the contrary, this is also true of the biometric tools.

J. ARTHUR HARRIS